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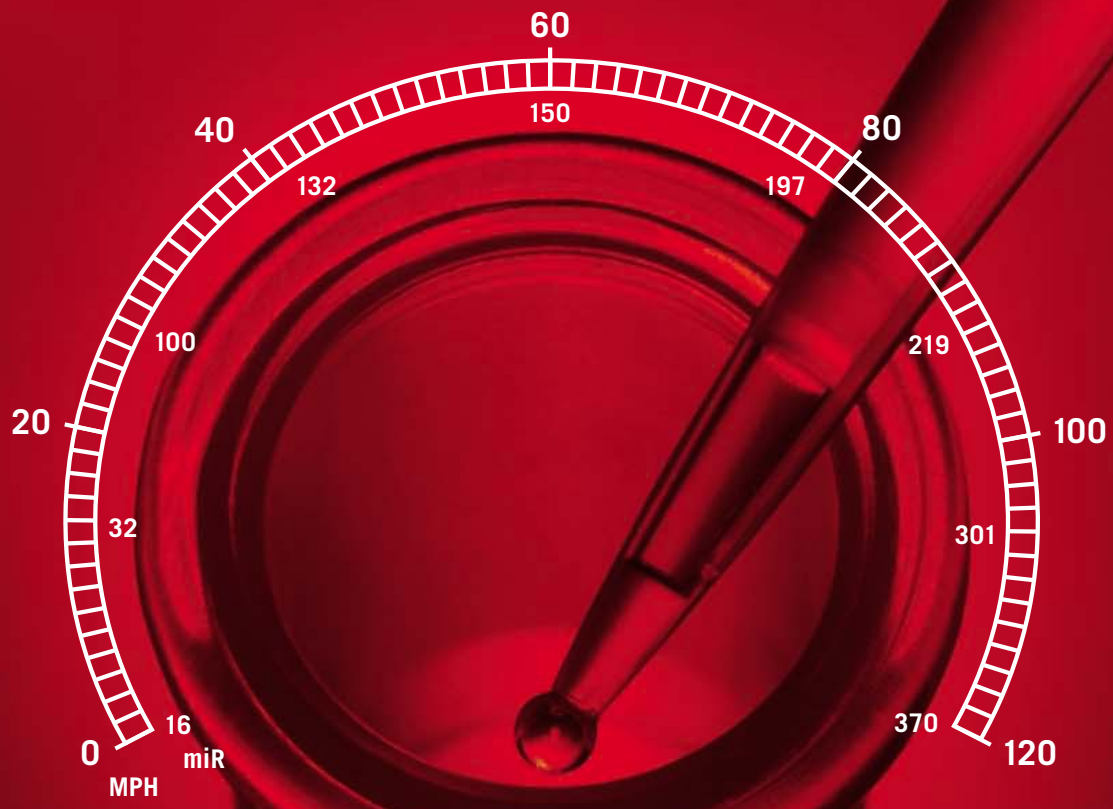

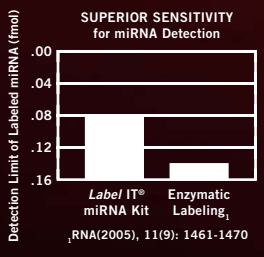

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SPECIAL ISSUE

GETTING ACROSS THE MEMBRANE

A variety of proteinaceous pores translocate ions, proteins, and DNA across cell membranes. A special section in this issue looks at how they accomplish this essential task. [Image: Chris Bickel]

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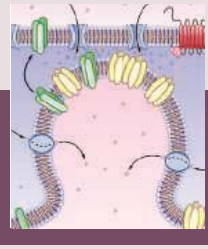

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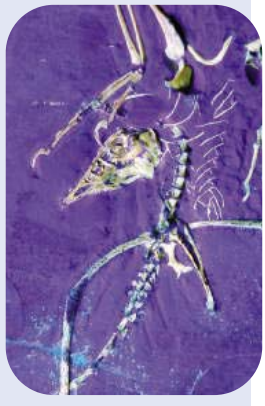

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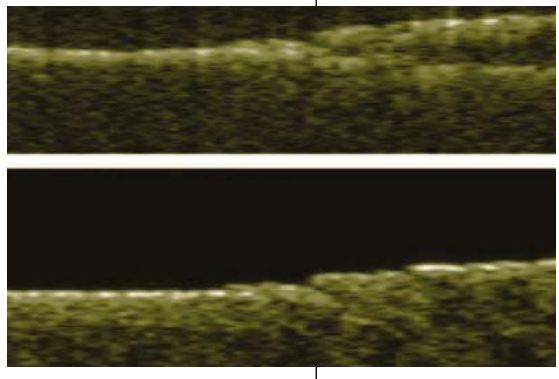

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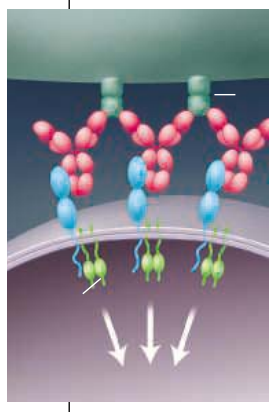

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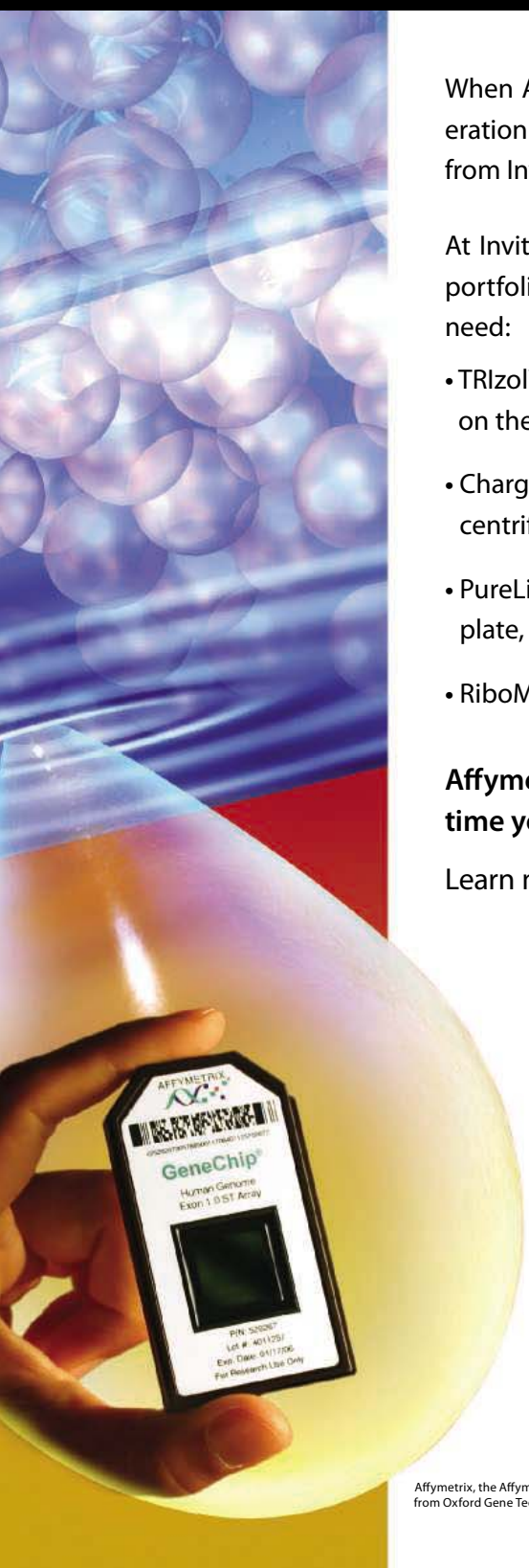
Opt for innovation



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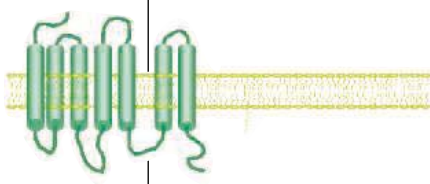

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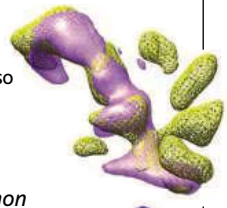

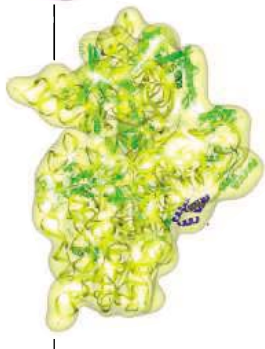

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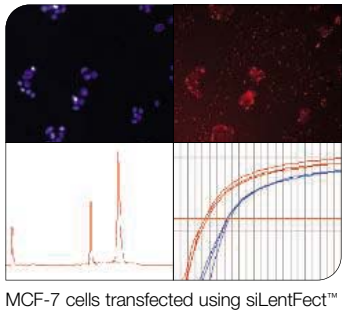

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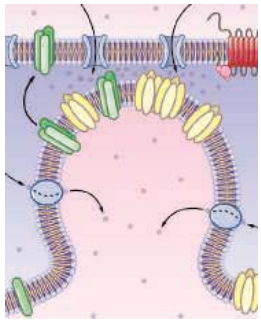

Signaling calcium influx.

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Dating Deep Circulation

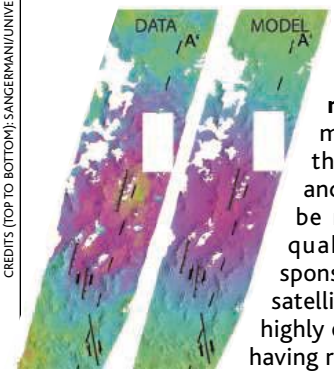
During the transition from the Last Glacial Maximum to the Holocene, a series of changes in the deep-ocean circulation pattern occurred in the North Atlantic. **Robinson *et al.*** (p. 1469, published online 3 November) made measurements of the carbon-14 content of the deep-sea coral *Desmophyllum dianthus* in order to characterize better the changes in circulation of intermediate and deep water in the North Atlantic during that transitional interval. The observed radiocarbon changes in the deep North Atlantic Ocean are consistent with the "bipolar seesaw" model of deep ocean circulation. The greater variability in waters at depths of less than 2500 meters correlates with smaller climate events that occurred near the poles.

Earlier Oxygen Onset?

Some microbes use the redox reactions of intermediate sulfur compounds as an energy source. These compounds originally formed via oxidation reactions, and thus it has been thought that these microbes evolved after about 1 billion years ago, when the oxygen content of Earth's atmosphere increased and caused a distinctive shift in the main sulfur isotopes ($^{34}S/^{32}S$) that was recorded in sediments. **Johnston *et al.*** (p. 1477) show that including data for ^{33}S isotope in the analysis provides a more accurate signal of microbial sulfur disproportionation. The diagnostic signal emerges considerably earlier than has been thought at about 1.3 billion years ago.

Long After the Quake

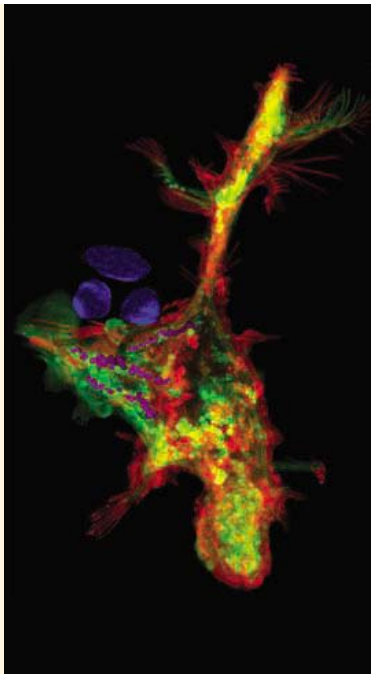
The extending western margin of the Great Basin is one of the more seismically active regions of North America, and four large earthquakes occurred in western Nevada from 1915 to 1954. **Gourmelen and Amelung** (p. 1473; see the Perspective by **Hammond**) used radar interferometry to map the continued deformation of this region during the past 10 years and show that the region still seems to be responding slowly to these earthquakes. Consideration of a broad response helps reconcile global positioning satellite data and imply that much of the highly extended crust to the east is now behaving rigidly.



CREDITS (TOP TO BOTTOM): SANGERMANI/UNIVERSITY OF QUEENSLAND; GOURMELEN AND AMELUNG

Expedient Cytokine Trafficking

Phagosomes are formed when cells such as macrophages engulf relatively large particles, like bacteria, from the external milieu. The source of membrane involved in the formation of the phagosome and the ability of other organelles to fuse with the phagosome is a topic of recent controversy. **Murray *et al.*** (p. 1492, published online 10 November) describe a fundamental and clever adaptation of phagosomal membrane trafficking in macrophages, whereby recycling endosomes fuse with the newly forming phagosome to create the site for release of tumor necrosis factor—a proinflammatory cytokine involved in innate immunity.



Mercurial Wetting

The interiors of carbon nanotubes can be filled by liquids through capillary action, but the surface tension of liquid metals such as mercury is too high for the metal to enter the nanotube by this process. Because of this lack of wetting, mercury has been used to form Ohmic contacts to carbon nanotubes. **Chen *et al.*** (p. 1480) present evidence for mercury entering open-ended, single-walled carbon nanotubes (SWNTs) by an electrowetting process that is facilitated by the potential drop created when the nanotube is used as a contact. Application of a bias potential changes the force needed to extract the SWNT from a mercury surface, and postmortem transmission electron microscopy indicates that mercury entered the interiors of the SWNTs and also wetted the exterior surfaces.

Bird Heads and Toes

Archaeopteryx is broadly recognized as the first known bird. It has been represented by nine specimens dating to about 150 million years ago (Late Jurassic). However, these nine specimens are all somewhat incomplete, particularly in important areas of the head and feet. **Mayr *et al.*** (p. 1483; see the news story by **Stokstad**) now describe a 10th specimen that shows new features in these important areas. Its first toe is only partially inverted, and its second can hyperextend. These features, as well as revealed parts of its skull, are notably similar to proposed theropod ancestors to birds.

Heading Off Hearing Impairment

Congenitally deaf cats and mice show clear abnormalities in the synaptic structure of auditory nerve endings. Are these abnormalities permanent, or could early treatment restore their original function? **Ryugo *et al.*** (p. 1490) compared normal hearing, congenitally deaf, and congenitally deaf cats fitted with a cochlear implant system. They investigated anatomical and functional restoration of the auditory nerve synapses; in particular, changes in a structure called the endbulb of Held. The artificial electrical stimulation of the cochlea by the cochlear implant rescued many of the normal features of this synapse.

The Matter of Taste

The sensation of taste is generated in taste buds, which then send the information through the gustatory nerves to the brain.

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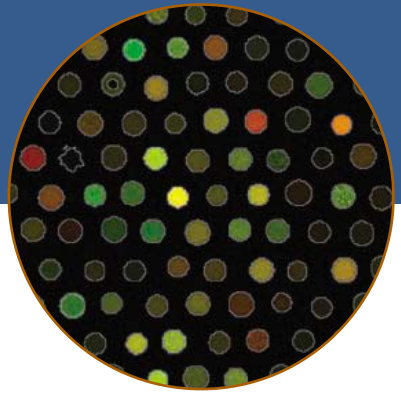

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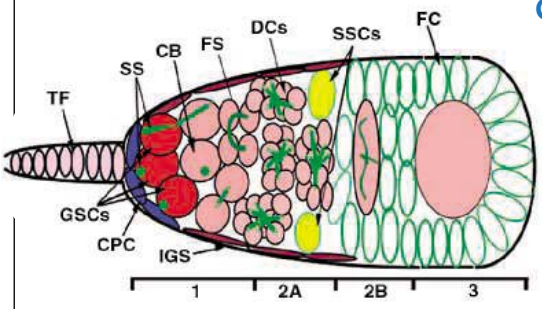
The neurotransmitter between the taste buds and the nerve had been thought to be serotonin, but mice genetically manipulated to lack functional serotonin receptors sense taste stimuli normally. **Finger *et al.*** (p. 1495) have investigated another candidate neurotransmitter that functions at these synapses, adenosine triphosphate (ATP). Mice lacking the two ionotropic receptors for ATP ($P2X_2$ and $P2X_3$) did not show responses to taste stimuli in the gustatory nerves. In addition, these mice could not detect most tastes in behavioral tests in which they had to show preference for one substance over another. These results, considered with the release of ATP from taste buds when they are stimulated, show that ATP is indeed the neurotransmitter at these synapses.



Chromatin and Stem Cells

Two stem cell types are found in the *Drosophila* ovary, germline stem cells and somatic stem cells. Self-renewal of these cells requires the function of the Hedgehog, bone morphogenic protein (BMP), and Wingless signaling pathways. **Xi and Xie** (p. 1487) now show that two adenosine triphosphate–dependent chromatin remodeling factors, Imitation SWI (ISWI) and DOMINO (DOM), also regulate self-renewal in the *Drosophila* ovary. DOM is required for somatic stem cell self-renewal and ISWI is required for germline stem cell self-renewal in response to BMP signaling in the stem cell microenvironment or "niche." Because this type of chromatin remodeling complex is highly conserved, it is likely that chromatin remodeling may play a role in stem cell self-renewal in other organisms.

Colon Cancer Connections

A previously unrecognized connection between two well-known signaling pathways appears to provide a crucial mechanism for control of proliferation of colon cancer cells. **Castellone *et al.*** (p. 1504, published online 17 November) show that the EP2 subtype of prostaglandin E2 receptor mounts a two-pronged attack that activates a transcriptional program that favors cell proliferation. When PGE2 binds to EP2, the associated heterotrimeric guanine nucleotide-binding protein (G protein) is activated. The G protein $\beta\gamma$ and α subunits act through distinct pathways that converge to promote stabilization and nuclear translocation of β-catenin, a protein that promotes transcription of specific genes that increase proliferation of cancer cells. This signaling system may explain why nonsteroidal anti-inflammatory drugs, which inhibit signaling through PGE2, can at times inhibit development of colon cancer in mice and human patients.

The IgGs Have It

Different classes of antibody (the immunoglobulins; IgA, IgD, IgE, IgG, and IgM) perform divergent functions within the immune system. IgG has also evolved further into subclasses that vary considerably in their potency in particular types of immune responses. Each IgG subclass possesses a range of binding affinities for the different inhibitory and activating receptors that engage the constant Fc region of the antibody molecule. **Nimmerjahn and Ravetch** (p. 1510; see the Perspective by **Woof**) used this observation to construct antibodies bearing the same antigenic specificity combined with the subclass-specific portions of Fc. The ability of these hybrid antibodies to mediate their immunological effects in vivo could be predicted by the strength with which the Fc portion bound the different activating or inhibitory Fc receptor (FcR). Thus, the specificity and strength of FcR binding is a central means by which IgG subclasses determine their dominance in a particular immune response.

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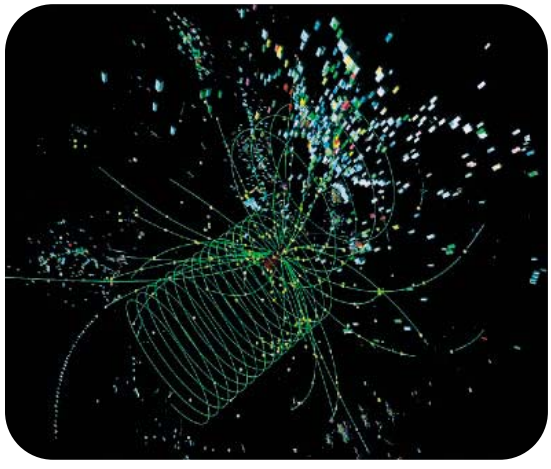
Bullish on Particles

Particle physics was, until recently, the flagship of U.S. physics, if not U.S. science. With ever larger "atom smashers" and such charismatic figures as J. Robert Oppenheimer and Richard Feynman, the field attracted the best and the brightest. These U.S. scientists garnered Nobel Prizes and public fame, becoming academic leaders and government advisors. The close association with national security that grew out of the Manhattan Project guaranteed both prominence and funding priority. But in 1993, the perfect storm hit: The $10 billion Superconducting Super Collider was canceled, the Cold War ended, and life sciences rose to prominence. Since then, we've seen flat budgets, more canceled projects, and no firm prospects for high-energy accelerator experiments on U.S. soil after 2009. In today's "flat world" where technology has made science around the world tightly interconnected, the future of particle physics everywhere can be no brighter than it is in the United States, and that future looks dark.



Despite this, I am bullish on the future of U.S. particle physics, and my reason is simple. Right now, the field is poised for breakthroughs as stunning as those that followed Einstein's *annus mirabilis* 100 years ago. The focus has shifted from searching for the smallest subatomic seed to understanding the universe and the nature of matter, energy, space, and time. Big questions are ripe for answering. What is the "dark matter" that holds our galaxy together? Where did space and time come from, and how many space-time dimensions are there? How did the universe begin, and what is the mysterious dark energy accelerating its expansion? And perhaps the biggest question of all, one whose answer probably underlies all the others: How are the two pillars of modern physics—quantum mechanics and general relativity—to be reconciled and a unified understanding of the forces of nature achieved? Particle physics is on the verge of something really big, as if the past 50 glory years were just preparation.

As exciting as these opportunities are, the challenges are great and morale in the U.S. particle physics community is low. With its link to national security severed, particle physics must now compete for funding and students with other fields that also have exciting agendas—from astrophysics and genomics to computer science and biophysics. Telescopes and underground laboratories to study dark energy and dark matter are now as essential as accelerators, making planning more complicated and the cost of discovery higher. And all of this in a time of constrained budgets for all science.

As a U.S. scientist, I can't imagine the United States not taking part in the grand scientific adventure ahead. Moreover, a reality of the flat world is that the field's big dreams will go unrealized if particle physics can't right itself in the United States. Three things are essential to correct the situation. If particle physics is to be successful in garnering the needed funding and attracting the best people, the field must lead with a broad scientific agenda, rather than defining itself by big atom-smashers as in the past. Hosting a $5 billon electron-positron linear collider to follow the Large Hadron Collider now being built in Geneva would bring high-energy physics back to the United States and make a strong statement of U.S. commitment to this field, but it must be the science, not merely the desire to reclaim the energy frontier, that dictates whether to push forward with such an endeavor. There must also be a commitment to diverse approaches. Recent discoveries (dark matter, dark energy, and neutrino mass) remind us that other tools are just as essential. Finally, particle physics must achieve unprecedented (for any field) global coordination. Many of the critical projects on the path to answering the big questions exceed the financial resources of any one country or region. A strong national presence must be balanced against a strategic global program. Not every facility can be located here, and a new strategy of U.S. leadership must replace the old strategy of U.S. dominance.

In their zeal to explore the world of the unimaginably small, particle physicists have repeatedly shown that they can blaze new trails and overcome formidable barriers. I am willing to bet that particle physicists in the United States and around the world will come through again. With unprecedented opportunities for revolutionary breakthroughs, all of science should be pulling for them.

Michael S. Turner

Michael S. Turner is Rauner Distinguished Service Professor at the University of Chicago and Assistant Director for Mathematical and Physical Sciences at the U.S. National Science Foundation.

10.1126/science.1121904

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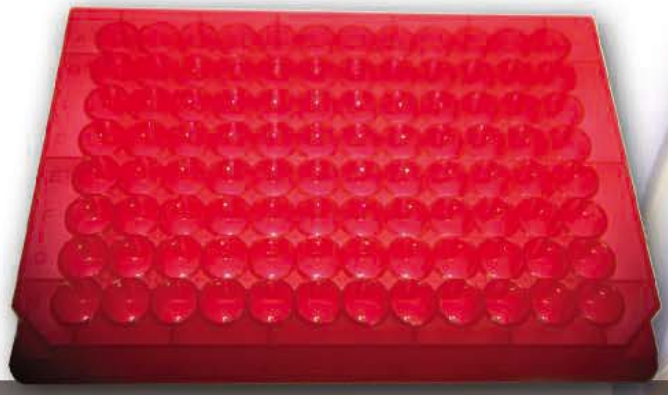

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ECOLOGY/EVOLUTION

A Hedging Strategy

The study site and *Senecio glaucus* (inset), one of the focal species.

Seed dormancy is a common adaptation in annual plants that live in highly seasonal or unpredictable habitats such as deserts. By delaying germination, plants can hope to escape conditions that are likely to be adverse for seedling growth. However, rather than germinating at once in response to a favorable cue such as rainfall, plants hedge their bets by varying the germination rates according to how reliably the cue predicts future conditions. In a study of annuals in the Negev desert, Tielbörger and Valleriani show that germination rates are higher for the relatively few seeds produced during dry years than for the large numbers of seeds produced in wet years, regardless of the abiotic cue. It appears that the plants predict the likelihood of future survival according to the density of seeds: a measure of the likely intensity of competition among seedlings. The authors suggest that information about the density of neighbors may be encoded in the seeds via maternal effects from the parent plant. — AMS

Oikos **111**, 235 (2005).

BIOMEDICINE

Gut Reactions

Celiac disease (CD) is caused by an immunological response to gluten peptides in wheat. This response damages the intestine and can compromise the absorption of essential nutrients. Specific variants of HLA class II genes (which encode proteins that participate in the immune recognition of gluten) confer an elevated risk of CD, but additional genes are likely to contribute to the disorder. Lifelong adherence to gluten-free diets is difficult, and there is interest in devising alternative therapies.

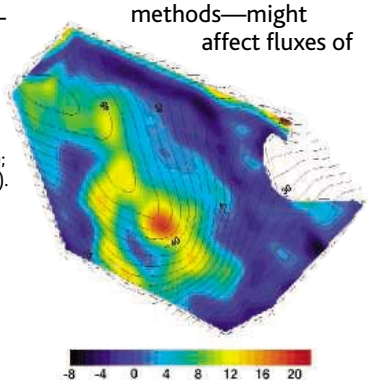
Promising new leads have emerged from genome-based studies of both the human victims and the plant assailant. In a genetic association analysis of two Dutch populations, Monsuur *et al.* identified a sequence variant that conferred a twofold greater risk of CD. This variant resides within an intron of the human *MYO9B* gene, which encodes an unconventional myosin that may play a role in the ability of intestinal epithelial cells to form a tight barrier, and the variant allele may increase the access of gluten peptides to immune cells. Spaenij-Dekking *et al.* investigated whether different varieties of wheat contain different levels of the gluten peptides that trigger the pathogenic immune reaction. Based on the results of database searches of gluten sequences and in vitro immunological assays, the authors concluded that sufficient genetic variation exists in wheat to warrant consideration of selection strategies that would produce varieties that are better tolerated by celiacs. — PAK

Nat. Genet. 10.1038/ng1680 (2005); *Gastroenterology* **129**, 797 (2005).

GEOCHEMISTRY

To Till or Not to Till

Soils contain approximately twice as much carbon as either land plants or the atmosphere. Because carbon is transferred so easily and quickly between soil and the air, how human activity might affect that transfer has important implications for the atmospheric carbon dioxide budget. Approximately 1.5 billion hectares (11% of the total land area of Earth) is cultivated, making the impact of agriculture on the concentration of atmospheric carbon dioxide potentially significant. A large debate has centered on how agricultural practices—whether the soil is tilled, a practice that accelerates the erosion of organic-rich topsoil, or cultivated using no-till methods—might affect fluxes of carbon between the land and the atmosphere.



Simulated soil redistribution at Saeby, Denmark (red, positive values indicate net flux to the soil).

Van Oost *et al.* use radionuclide and soil organic carbon data to analyze the fate of sediment and soil organic carbon during erosion and deposition in agricultural uplands. They find that, contrary to earlier studies, which did not include depositional processes, agricultural uplands can experience a net gain of carbon by the formation of new soil organic carbon at eroding sites and the burial of eroded soil organic carbon below plough depth. Thus, rather than causing a net carbon loss, tillage might be an important mechanism for carbon sequestration in certain cases. — HJS

Global Biogeochem. Cycles **19**, 10.1029/2005GB002471 (2005).

MATERIAL SCIENCE

Peak Growth

There is wide interest in fabricating large, defect-free, three-dimensional periodic crystals for use in photonic applications. One simple method involves the growth of colloidal crystals; however, most such methods produce crystals with stacking faults and macroscopic cracks. The defects arise in part because the difference in free energy between the face-centered cubic and hexagonal close-packed structures is small.

Jin *et al.* found that by reducing the growth temperature from 65° to 24°C and by decreasing the concentration of particles in solution, they were able to grow crystals with both the (111) and the more desirable but less energetically favorable (100) orientations on a flat substrate. They explored the role of templating the substrate by building pillars of hydrogen silsesquioxane with spacings of 308 to 320 nm, on which they grew crystals with a

CONTINUED ON PAGE 1393

CREDITS: (TOP) KATJA TIELBÖERGER; (BOTTOM) VAN OOST *ET AL.*, *GLOBAL BIOGEOCHEM. CYCLES* **19**, 10.1029/2005GB002471 (2005)

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particle diameter of 299 nm. By vastly slowing down the growth rate and tilting the substrate, they obtained crystals that were free of cracks and faults, although there was the odd defect where differently sized colloidal particles were located. The crack-free nature of the crystals is due to the underlying template, which forces the bottom layer of particles to take on a non–close-packed arrangement, giving the particles a bit of space to move about as the crystal grows and dries. — MSL

Nano Lett. 10.1021/nl051905j (2005).

IMMUNOLOGY

Helpful Helminths

Pathogens have evolved countless devious means of thriving within their hosts. These range from antigenic escape from the attention of B and T cells to usurping the early detection network of the innate immune system.

Wilson *et al*. provide evidence to suggest that the nematode gut parasite *Heligmosomoides polygyrus* protects itself by suppressing allergic T cell responses in the host. Nematode infection was found to decrease the pulmonary allergic inflammation normally evoked in mice by an allergen from the house dust mite. Tying several lines of evidence together, the effects were narrowed to a population of regulatory $CD4^+$ T cells from gut-associated lymph nodes of infected mice. Smith *et al*. found that another helminth, the trematode parasite *Schistosoma mansoni*, produces a chemokine-binding protein (CKBP) to protect itself from the ill effects of host inflammation. CKBP was detected specifically in the egg stage of the parasite and bound CXCL8 (IL-8) and CCL3 (MIP1a). Predominantly through effects on neutrophil activity, CKBP inhibited different forms of experimental inflammation in mice. Both studies reveal a new layer of diversity by which helminths modify their host environment. — SJS

J. Exp. Med. **202**, 1199; 1319 (2005).

BIOCHEMISTRY

Impedance Matching

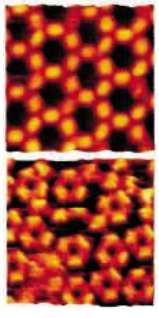
The current vogue for treating metabolic and regulatory pathways as circuits in which parts can be swapped in and out, with sensors at the input side and cellular behavior at the output side, has been driven by the ability to construct sensors by modifying natural ligand-binding receptors and to insert heterologous genetically coded components. Invasin is a cell-surface protein of *Yersinia pseudotuberculosis* that initiates bacterial uptake by binding to integrin, a protein on the surface of some mammalian cells, and previous work has shown that transferring the *inv* gene into *Escherichia coli* is sufficient to enable it to invade integrin-expressing cells. Anderson *et al*. have engineered *E. coli* in which *inv* is under the control of the promoter from *fdhF*, a gene whose expression is induced by hypoxia (one characteristic of tumor microenvironments). They discovered that in order to dial down the basal level of *inv* expression in their construct, it was necessary to etiolate the wild-type ribosome-binding site by randomizing flanking bases in a library of 10^6 members and screening for the handful of clones in which sensor input and behavioral output were matched so as to support a strictly anaerobic-dependent invasion. — GJC

J. Mol. Biol. 10.1016/j.jmb.2005.10.076 (2005).

SURFACE CHEMISTRY

Heat and Meet

The formation of well-ordered supramolecular arrays on metal surfaces by large molecules is favored by high surface mobility and strong molecular interactions, requirements that work at cross purposes. Stöhr *et al*. show that a large perylene derivative, DPDI (4,9-diaminoperylene-quinone-3,10-diimine), does not form hydrogen bonds at room temperature on an atomically flat Cu(111) surface, but does after annealing at 300°C, which causes the loss of H_2 and converts some of the amino groups into hydrogen bond acceptors. Scanning tunneling microscopy (STM) revealed the formation of open honeycomb networks for surface coverages of DPDI between 0.1 and 0.7 monolayer (ML) after high-temperature annealing; above 0.7 ML, the honeycomb structure occupied too much area, and at 0.85 ML, trimers formed instead. Finally, at 1 ML, chained structures that minimize the space between molecules formed. — PDS



STM images (0.1 to 0.7 ML, upper; 0.85, lower) of DPDI aggregates.

Angew. Chem. Int. Ed. **44**, 7394 (2005).

CREDITS: STÖHR *ET AL.*, *ANGEW. CHEM. INT. ED.* **44**, 7394 (2005)

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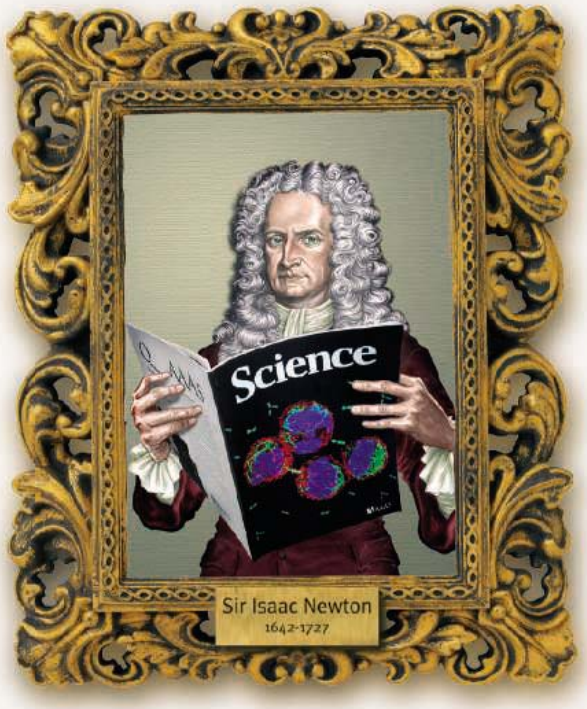

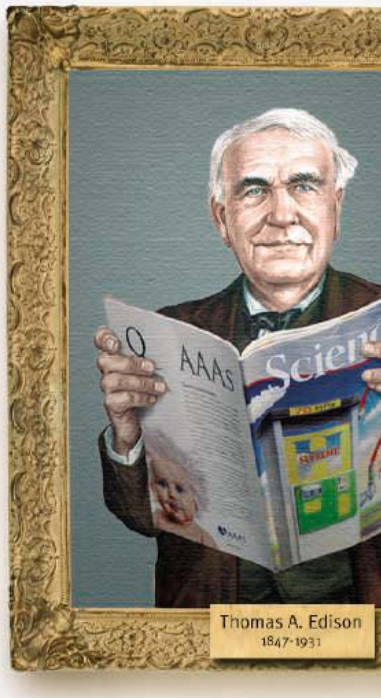

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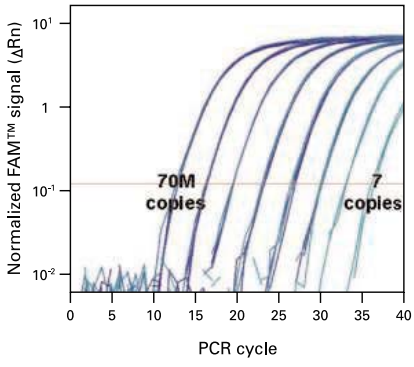

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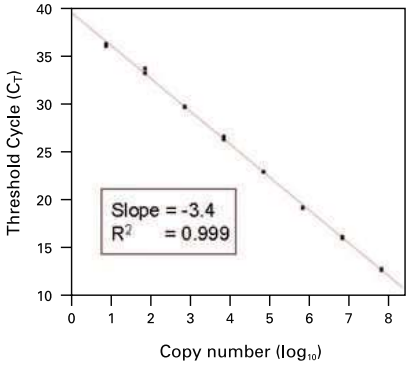

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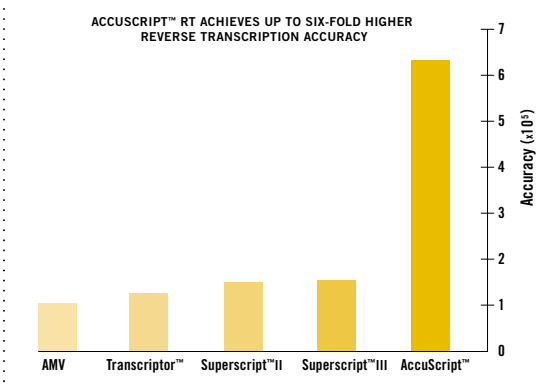

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1. Roberts, J.D., Bebenek, K., Kunkel T.A. The Accuracy of Reverse Transcriptase from HIV-1. Science 1988 (242) 1171-1173.

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NetWatch

edited by Mitch Leslie

EDUCATION

Worldly Analysis

High school students and undergraduates who work through the Earth Exploration Toolbook get the chance to crunch actual data from NASA, the U.S. Geological Survey, and other sources. Hosted by Carleton College in Northfield, Minnesota, the tool book features 13 chapters written by teachers and researchers that tackle timely earth science questions. Detailed instructions guide students through each procedure as they use satellite measurements to trace changes in the size of the Antarctic ozone hole, for example, or apply ocean buoy data to predict where phytoplankton blooms will erupt in the Gulf of Maine.

serc.carleton.edu/eet

RESOURCES

Puffballs and Morels And Rusts, Oh My!

You might find the gelatinous fungus known as witch's butter (*Dacrymyces palmatus*; left) protruding from cracks in the bark of pine trees. To learn more about the habitats, structure, and reproduction of witch's butter and other fungi, dig into MykoWeb* from computer consultant Michael Wood of San Leandro, California. Aimed at researchers and amateur mushroom fans, the site reprints a classic mycology text and features articles from experts on topics such as the latest taxonomy and the biology of mycorrhizae, the partnerships between plant roots and fungi. But the centerpiece of MykoWeb is California Fungi, a photo-packed guide to more than 400 of the state's species, including *D. palmatus*.

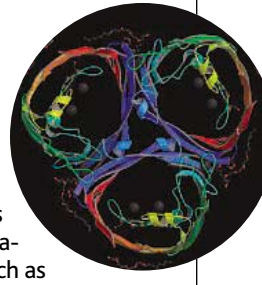
To check on species that dwell farther north, visit The Pacific Northwest Fungi Database† from Washington State University in Pullman. The growing site catalogs some 5000 types of fungi. Listings include the species' classification, who first described it, and the original reference.

* www.mykoweb.com

† pnwfungi.wsu.edu/programs/aboutDatabase.asp

DATABASE

Proteins on the Edge



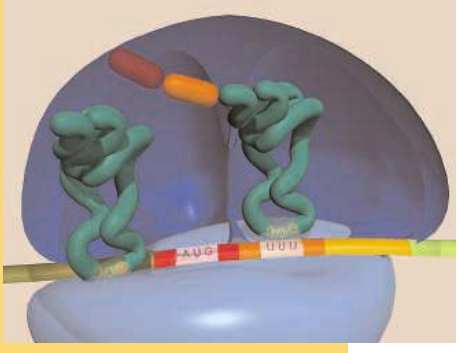
Membrane proteins connect cells to their environment, shuttling materials in and out and picking up communiqués from other cells. At this new clearinghouse run by structural biologist Martin Caffrey of Ohio State University in Columbus and colleagues, you can get the lowdown on more than 140 of these proteins, which are embedded in membranes or positioned near them. The site's profiles summarize information gleaned from other collections such as the Protein Data Bank and from the literature. Pick a molecule such as porin (right), which allows bacteria to sop up ions and nutrients, to uncover structural details such as how many times it winds through the membrane (16) and whether it harbors any metals or other nonprotein components (no). The entries also summarize how researchers crystallized the protein and determined its architecture.

www.lipidat.chemistry.ohio-state.edu/MPDB/index.asp

IMAGES

Molecules in Motion

A transfer RNA molecule hands off its amino acid to a growing peptide strand dangling from another transfer RNA (below). The relay is a key maneuver in protein synthesis, or translation. High school and college students can follow the steps of translation or zoom in on other biological processes at the Virtual Cell Animation Collection from North Dakota State University in Fargo. Playing at the site are eight narrated animations that show how protons trickling through the mitochondrial membrane power ATP synthesis, for example, and illustrate which segments get chopped out during mRNA splicing. Beginners who only need an overview of the action can click through the stills in the "First Look" sections. The "Advanced Look" options provide more details for upper-division college or grad students.



vcell.ndsu.nodak.edu/animations

TOOLS

The Biomedical Literary Companion

You've found a PubMed abstract for a paper by A. Chen and want to track down other publications by the same author. You could try winnowing the more than 1500 hits on that name, or you could click over to the Arrowsmith Project Web site from neuroscientist Neil Smalheiser of the University of Illinois, Chicago, and colleagues. The project's "Author-ity" tool weighs criteria such as researcher affiliation, co-author names, journal title, and medical subject headings to identify the papers most likely written by your chosen scientist. The site offers other helpers for squeezing information out of PubMed results, such as the Arrowsmith feature, which pinpoints common terms in two lists of search results.

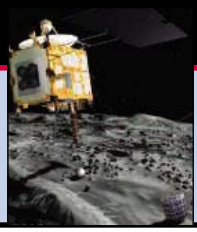
arrowsmith.psych.uic.edu/arrowsmith_uic/index.html

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): MICHAEL WOOD; PROTEIN DATA BANK; VIRTUAL CELL ANIMATION COLLECTION

This Week

PAGE 1406
Reprocessing revival?



1409
No going home again?

STEM CELL RESEARCH

Korean Cloner Admits Lying About Oocyte Donations

South Korea's ambitious plans to create a World Stem Cell Hub, announced in October, were thrown into uncertainty on Thanksgiving Day when Korean researcher Woo-Suk Hwang resigned as president of the venture and from other official posts. He remains a researcher at Seoul National University. Hwang acknowledged in an emotional press conference that two researchers in his lab had donated eggs for his research, and that donors had been paid for their contributions—something he had denied for months.

The admission seems to have done little to diminish Hwang's support in Korea, where he has enjoyed rock-star status, including an "I Love Hwang Woo-Suk" fan club (cafe.daum.net/ilovehws). Colleagues have reportedly urged Hwang to stay on as leader of the country's bold bid at world leadership in stem cell research. Korean newspapers and Web sites report that sponsors are pulling ads from a TV program that uncovered alleged irregularities in Hwang's egg-collection methods, and Korean women are lining up to donate eggs for stem cell research: A group set up on 21 November to encourage egg donations (www.ovadonation.or.kr) had been contacted by 800 would-be donors by the end of the week, according to a spokesperson.

Buoyed by the outpouring of public support, Hwang told *Science* in an e-mail that he's "considering reconsidering" his resignation. But such a turnaround seems unlikely as repercussions ripple through the global community of stem cell researchers. Most scientists would probably agree with bioethicist Insoo Hyun of Case Western Reserve University in Cleveland, Ohio, that "he did the right thing by stepping down."

The events that led to Hwang's downfall appear to be limited to the landmark paper he published in *Science* early last year announcing the world's first success in cultivating a line of stem cells from a cloned human embryo (*Science*, 12 March 2004, p. 1669). The consent form, summarized in supporting online materials, said the 16 donors had received "no financial payment" for the 242 eggs they contributed to the experiments, although such payments would have been legal under Korean law at the time. (*Science* Editor-in-Chief Donald Kennedy says a correction will be published.)

Some activists and bioethicists wondered how Hwang's team could have located so many willing egg donors. Then in May 2004, *Nature* reported that one of Hwang's Ph.D. students, a co-author of the paper, had said in an interview that she and another lab member had donated eggs. Such donations would be ethically questionable because students may feel pressure to donate. The student later denied it, however, pleading poor English skills, and Hwang denied that anyone from his lab had donated eggs.

A very public apology. Woo-Suk Hwang's press conference prompted an outpouring of support in Korea but a more negative reaction elsewhere.

Rumors about possible improprieties in egg donations heated up again this fall after the 19 October unveiling of the World Stem Cell Hub based at Seoul National University. Hwang's denials began to unravel on 11 November, when his most prominent U.S. collaborator, Gerald Schatten of the University of Pittsburgh in Pennsylvania, announced that he was severing ties with Hwang, claiming that Hwang had misled him (*Science*, 18 November, p. 1100). Ten days later, Sung-Il Roh, who runs a fertility clinic at MizMedi Hospital in Seoul that supplied eggs for Hwang's research, announced that he had paid at least 20 women about $1430 each for eggs he had furnished for the 2004 study. Roh said the collections occurred in 2002, before Korea passed a law making such payments illegal. As Hwang's work became well-known, Roh said women were willing to donate eggs without compensation. Roh insisted that Hwang did not know of the early payments.

Hwang finally came clean last week. He admitted that after receiving a call from *Nature* last year, he asked the two women if they had donated eggs. They confessed but "begged me not to publicize the fact" to preserve their privacy. "Now that I reflect on it," he said, "I regret that I didn't come out with the truth." As for payments to donors, he said, "I only found out that some of those eggs had been paid for when Dr. Roh called me a few days ago."

The revelations prompted the ruling party in South Korea's National Assembly to announce plans to set up a new group to ponder bioethics, and the Korean Bioethics Association convened a meeting to discuss what occurred in Hwang's lab. The institutional review board of Seoul National University's veterinary college also investigated the controversy and recommended that a third party with global credibility examine the matter.

Elsewhere in Asia, researchers are feeling the ripples. Norio Nakatsuji, a stem cell researcher at Kyoto University, worries that the fallout could affect discussions on government guidelines for human embryonic stem (ES) cell research, which he fears "may become more strict because of this event."

Arnold Kriegstein, head of the Institute of Tissue and Stem Cell Biology at the University of California, San Francisco (UCSF), says creation of the World Stem Cell Hub may have been "premature." He says hub officials approached UCSF as a possible location for one of the two planned subhubs for generating new lines of human ES cells. But Kriegstein says that after meeting with Hwang's delegation, "we decided not to participate," mainly because guidelines were ▶

CREDIT: IM JAE-HWAN/AFP/GETTY IMAGES

unclear on ethical issues such as consent forms for egg donors and the tracking of research materials. Kriegstein and others are not writing off collaboration with the Koreans, however, and they acknowledge that Hwang's published findings are not in doubt. "It's not a blow to the field but to him personally," says Kriegstein.

In the United Kingdom, scientists have generally voiced sorrow about Hwang's mistake and pride in their own system of safeguards. "This highlights why the tough regulatory climate in the U.K. is protection rather than a problem," said biologist Steven Minger of King's College in London.

The future of the hub is now uncertain. On 15 November, the Korean government laid out plans to invest 11.5 billion won ($11 million) in the venture and make it independent from Seoul National University. There will be no subhub in San Francisco, at least for now. It has been rebuffed by both UCSF and the new California Institute for Regenerative Medicine. And the San Francisco–based Pacific Fertility Clinic, which had agreed to help with egg collection, said last week that it had severed ties with Hwang. Ian Wilmut of the University of Edinburgh, which the hub was eyeing as its European outpost, said "we are saddened" by the events, but "I hope that we can develop collaborative links" with the Koreans.

Ironically, some maintain that Hwang now has an operation second to none in its ethical safeguards. This week, *The American Journal of Ethics* published an article by Hyun describing in detail the guidelines now used by Hwang's group for egg procurement, along with a commentary by Mildred Cho and David Magnus of Stanford University in Palo Alto, California, who say that if the outlined procedure is followed, it is "a major step toward meeting the highest standards of ethical oversight for oocyte donation." **–CONSTANCE HOLDEN**

With reporting by Gretchen Vogel and Dennis Normile.

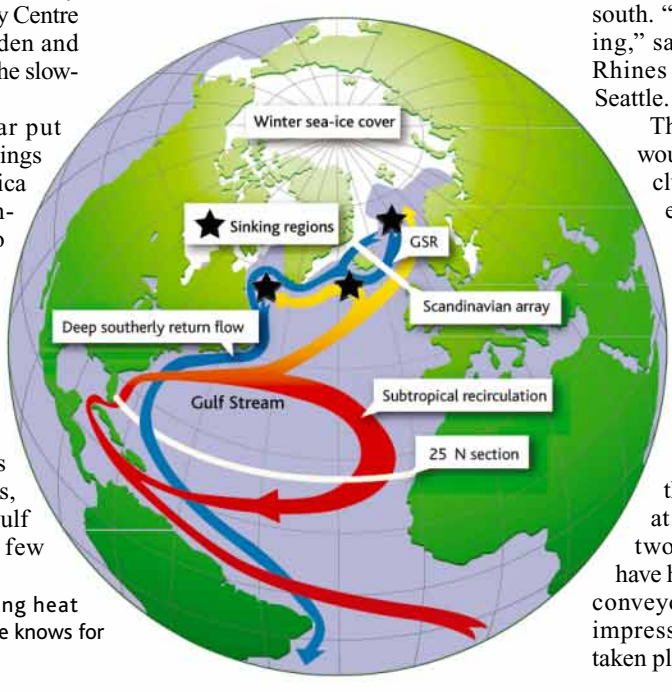
GLOBAL CLIMATE CHANGE

The Atlantic Conveyor May Have Slowed, But Don't Panic Yet

The ponderous churning of the North Atlantic Ocean that carries warm water northward and returns deep, cold water to the south appears to have slowed in the past decade or two. That would mean that this oceanic radiator is bringing less heat to warm Europe and, if global warming is behind the slowdown, will carry less and less heat to high latitudes in the future. But the slowing is hardly larger than the uncertainty of the observations. And "we don't know enough about the ocean to know whether this represents a trend" that will persist, says physical oceanographer Harry Bryden of the National Oceanography Centre (NOC) in Southampton, U.K. Bryden and NOC colleagues report detection of the slowdown this week in *Nature*.

Oceanographers only last year put down a string of instrumented moorings spanning the Atlantic from West Africa to the Bahamas, so for a long conveyor record, the NOC group had to draw on five oceanographic surveys across that stretch of the Atlantic between 1957 and 2004. During ship crossings of a month or two, researchers measured seawater temperature and salinity from the surface to near the bottom. The NOC group used seawater densities calculated from those observations, plus current measurements of the Gulf Stream passing by Florida and a few standard assumptions, to estimate the currents heading north and south through the depth of the Atlantic.

The Gulf Stream remained steady through the 47-year period, and Atlantic flows remained much the same through the 1992 survey. But according to the NOC group's analysis, the conveyor appears to have slowed dramatically in 1998 and 2004. Fifty percent more Gulf Stream near-surface waters were turning back southward before reaching very far to the north, whereas part of the deep southward flow of cold water had decreased by 50%. All in all, the conveyor had slowed by 30%.



A slowdown? Currents (red) carrying heat northward may have slowed, but no one knows for how long.

CREDIT: DETLEF QUADFASEL, *NATURE* **438**, 565 (2005)

The slowing, although sizable, is comparable to the estimated uncertainty of the observations, Bryden notes. Still, "it's real variability," he says. Observed temperature changes driving the conveyor slowdown in shallower waters in the west and in deeper waters are just what he would expect from salinity and circulation changes previously reported in the far north (*Science*, 16 April 2004, p. 371). That's where the conveyor turns down from the surface and heads back south. "The pattern is reasonably convincing," says physical oceanographer Peter Rhines of the University of Washington, Seattle. "It's a pretty nice picture."

The picture is still fuzzy, however. "It would be dangerous to jump to the conclusion that there's a persistent weakening" of the conveyor circulation, says ocean and climate modeler Richard Wood of the Hadley Centre for Climate Prediction and Research in Exeter, U.K. Wood, Rhines, and Bryden all worry that the near-instantaneous snapshots taken by the ocean surveys might have been misleading. Like any part of the complex climate system, the conveyor is bound to slow down at times and speed up at others. The two latest surveys, Wood says, may have happened to catch the Atlantic as the conveyor slowed temporarily, giving the impression that a permanent change had taken place. ▶

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On the other hand, the NOC analysis may not have even captured what happened in the past decade or so. Climate models simulating the conveyor in a warming world don't call for such a large slowdown until sometime in the next century, Wood notes. In fact, climate researcher Jeff Knight of the Hadley Centre and colleagues recently reported that changing sea surface temperatures suggest that the conveyor has speeded up a bit since the 1970s (*Science*, 1 July, p. 41). And physical oceanographers Carl Wunsch and Patrick Heimbach of the Massachusetts Institute of Technology have just crunched far more oceanographic data from a variety of sources over the interval of dramatic change (1993 to 2004) in the NOC analysis. In a paper submitted for publication, they report a small slowdown, a quarter the size of the NOC group's. The change in heat transported northward is negligible, they calculate.

So has the conveyor slowed? Might it continue to slow? "We don't know," says Wunsch. And it may take a decade or two more of watching and waiting to know for sure.

–RICHARD A. KERR

CANADA

Animal Rules Keep Grad Students Out of the Lab

OTTAWA—Twenty graduate students are suing Laurentian University in Sudbury, Canada, this week in hopes of regaining access to their rat labs. The students are caught in the middle of a dispute between their high-profile adviser, neuropsychologist Michael Persinger, and the institution over Persinger's compliance with animal-welfare rules, a battle that his supporters say is fueled by the university's desire to make room for its new medical school.

Laurentian's associate vice-president (research) Liette Vasseur says that the school changed old locks on 9 November and didn't give new keys to the students because the university's animal-welfare committee had not approved seven of Persinger's research protocols. Until the protocols are approved, the experiments cannot be performed, and the students "have no need to go in the building if they don't do research there," says Vasseur.

Rats! Laurentian's Martin Persinger and graduate students are fighting the university's animal-care policies.

CREDIT: LINDA ST. PIERRE

But that explanation didn't placate the students. "You don't come in and just lock doors. That's draconian and barbaric," says Robert Brouillette, lawyer for the students, who since 9 November have been unable to conduct various obesity, epilepsy, cancer, aging, and behavioral studies under the unapproved protocols.

Persinger is considered a maverick for work on inducing religious or quasi-mystical states in human patients via magnetic stimulation of their left temporal lobes. In addition to that research, in a field tabbed neurotheology, he and his students use some 800 rats for a variety of experiments that occupy 70% of the school's animal-care facility.

The dispute between Persinger's group and Laurentian's animal-care committee involves the interpretation of two Canadian Council on Animal Care (CCAC) requirements: that rats be housed in plastic rather than steel cages and that they be euthanized at the end of an experiment. CCAC Executive Director Clement Gauthier says rats in steel units develop lesions on their feet and that euthanasia is recommended if experiments are invasive or yield "chronically ill" rodents. But Persinger says he's not convinced that plastic cages are better—one of his studies compares the long-term effects on animals of plastic and wire cages—and he believes that it's more humane and appropriate to treat sick rats after the experiment. Ian Duncan, chair of animal welfare at the University of Guelph, says neither plastic cages nor euthanasia are obligatory and that there can be sound reasons for alternative practices.

The battle escalated this fall, say observers, when Laurentian became the country's 17th medical school. That expansion prompted an agreement with the nation's three granting councils promising full compliance with CCAC guidelines and increased the pressure for space on the university's limited animal-care facilities.

But Persinger says the university has negotiated in bad faith. "You answer this and this and then they come back, well, now you need that and that," he says. "It's an infinite progression."

–WAYNE KONDRO

Wayne Kondro writes from Ottawa, Canada.

ScienceScope

No to GMO, Say Swiss

Pharma and agrochemical companies may "take their research out of the country" now that growing genetically modified (GM) plants is illegal in Switzerland, says Bernd Schips, director of the Swiss Institute for Business Cycle Research. This week, Swiss voters approved a 5-year moratorium on the cultivation of GM organisms (GMOs) and the import of transgenic animals. GM field trials and GM food imports are still permitted. "[We] hope Switzerland's rejection of [GM] crops inspires others around the world to ... say no," said Greenpeace's Geert Ritsema. Swiss agbiotech firm Syngenta said its U.S.-based GMO research would be unaffected.

–XAVIER BOSCH

NSF Gender Snoops on Campus

The National Science Foundation is investigating four institutions that receive NSF research funds to determine whether they are in violation of Title IX—the law that prohibits sex discrimination by any school receiving federal dollars. The move follows a 2004 Government Accountability Office report that charged NSF and two other science agencies with failing to track Title IX compliance.

The agency declined to disclose the sites being investigated. Chemist Debra Rolison of the Naval Research Laboratory in Washington, D.C., calls the reviews a "good first step" toward using Title IX to improve the gender ratio in technical fields, in the same way that the law has transformed college athletics.

–YUDHIJIT BHATTACHARJEE

Researchers Get Hippocratic

Scientists must do no harm, say 68 of the world's science academies. The InterAcademy Panel on International Issues this week released principles for drafting codes of conduct at biology labs. The panel's statement on biosecurity recommends that scientists refuse to do research deemed "only harmful," take steps to "secure" laboratories, and report activities that violate the 1972 Biological and Toxin Weapons Convention. "Do no harm is an excellent starting point," says bioweapons expert Ronald Atlas of the University of Louisville, Kentucky, but he acknowledges that certain provisions will be controversial. A call for whistleblowing, for example, could raise questions about where researchers should report violations and how they will be protected, he says.

–MICHAEL SCHIRBER

NUCLEAR POWER

Congress Tells DOE to Take Fresh Look At Recycling Spent Reactor Fuel

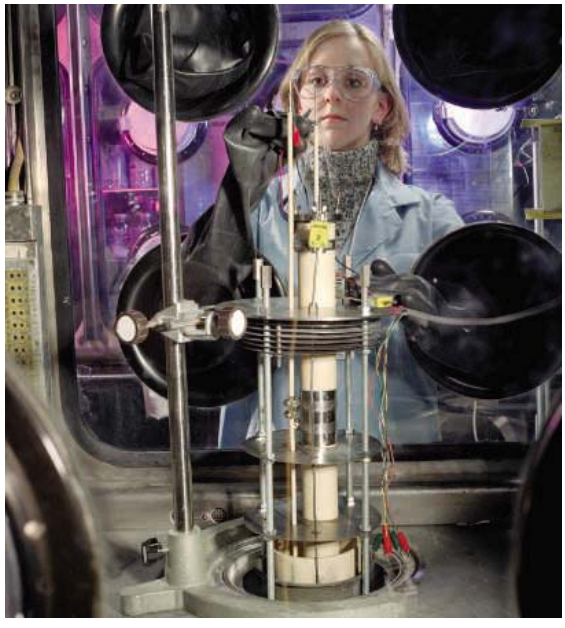
The United States is laying plans that could lead to recycling commercial nuclear waste into fuel for the first time in almost 30 years. But critics worry that such a boost for nuclear power could undermine global efforts to stop the spread of nuclear weapons.

The Department of Energy's (DOE's) new budget, signed by President George W. Bush last month, contains $50 million toward a goal of beginning construction on an engineering-scale reprocessing plant by 2010. Supporters say that recycling fuel could not only save time and money but also ease a mounting nuclear waste problem. Opponents dispute each of those points, adding that the technology needed is not yet at hand and that the United States, by recycling waste, would be sending the wrong signal to the rest of the world.

Researchers have explored reprocessing spent nuclear fuel rods since the dawn of the nuclear age. U.S. government officials pushed recycling commercial fuel in the 1960s when uranium was thought to be scarce and plutonium was considered a good fuel. Separating out the plutonium and uranium from other fissionable material also would reduce quantities of certain types of highly radioactive nuclear waste, thus in theory increasing the storage potential at the yet-to-be-built Yucca Mountain repository in Nevada. "The pursuit of [safe] recycling technologies … must be considered not just a worthwhile but a necessary goal," DOE Secretary Samuel Bodman said earlier this month.

But plutonium is also used in nuclear weapons, and critics say that producing more of it increases the likelihood that some will get into the wrong hands. The United Kingdom, France, and Japan use an aqueous method to recover uranium and plutonium from spent fuel rods. That technique, called PUREX, involves dissolving the rods with acid and chemically separating the two fuels. Japanese scientists have found that the approach is not economically viable, and the French experience has been mixed. Supporters also say reprocessing could forestall construction of an expensive second storage facility if, as projected, Yucca runs out of space within a decade—assuming the facility overcomes legal barriers to open.

With the growing interest in nuclear energy as an alternative to greenhouse gas–emitting technologies, scientists have developed advanced reprocessing techniques aimed at solving the waste issue without adding to the proliferation threat. One experimental approach, touted by scientists at DOE's Argonne National Laboratory in Illinois, is to use aqueous methods similar to PUREX with extra chemical steps to keep plutonium mixed with uranium and to retain nasty fission products that make the product too radioactive to steal. Another method, called pyroprocessing, employs electrochemistry to create a metal fuel that could include a fission product called cerium-144, which remains highly radioactive for 2 years. The fuel, which would be hot and therefore tough for thieves to handle, could theoretically be fed immediately into an adjacent reactor to provide power, say advocates. Argonne deputy associate lab director Phillip Finck says that radiation monitors and tight security could make both recycling methods proliferation-resistant.



Reduce, reuse, recycle? Argonne's Laurel Barnes studies a nuclear fuel reprocessing technique that converts oxide fuel to metal.

CREDIT: ARGONNE NATIONAL LABORATORY

But Princeton University physicist Frank von Hippel and others dispute the advantages. Most U.S. spent fuel is about 20 years old, he points out, making the nonproliferation advantages of cerium in pyroprocessing "irrelevant for the spent fuel we have." Monitoring techniques to keep track of plutonium in a complex facility are woefully inadequate, says Edwin Lyman of the Union of Concerned Scientists in Cambridge, Massachusetts. Moreover, said Representative Edward Markey (D–MA) during a House debate in May, the current ban on reprocessing nuclear fuel "gives us the high moral ground as we look at the North Koreans and Iranians to tell them not to do it." In 1977, President Jimmy Carter halted federal support for commercial recycling after India used civilian reprocessing to obtain nuclear weapons.

Experts say the technology is likely to remain prohibitively expensive. A 1996 National Research Council study found that recycling existing U.S. spent fuel rods could cost up to $100 billion; building the fast reactors to burn recycled fuel obtained by pyroprocessing or by advanced methods would be a major element of that cost. A 2003 study by researchers at Harvard University and the University of Maryland found that reprocessing uranium using current industrial methods would be economical only if the cost of obtaining uranium were to increase by a factor of 10. Geologists have only recently begun to look for new sources, but former Argonne reprocessing specialist Milt Levenson says the price could soon rise if demand increases—although he says there are too many factors at play to make an economic argument for or against reprocessing.

Reprocessing could cut storage costs by keeping very-long-lasting isotopes in the fuel cycle, say supporters, allowing DOE to store the fission products with less long-term heat more compactly within Yucca. The Yucca repository is designed to store spent fuel rods in dry casks for 10,000 years. Opponents of reprocessing would prefer that U.S. utilities continue to follow that course—and that Congress expand Yucca only after exploring aboveground storage for fuel rods. Research on advanced recycling should continue, they add, but not at the risk of undermining diplomatic efforts to stop reprocessing abroad. If recycling methods show promise down the road, they say, spent fuel could be retrieved from Yucca and tapped for power. "We don't need to do it now. We don't have the technical knowledge to do it now," says physics Nobelist Burt Richter, a member of an American Physical Society technical committee that in May called for a cautious approach.

But growing energy demands require more nuclear plants, say supporters, and the waste problem needs reprocessing. "The federal government does a lot that isn't economical," says Representative Judy Biggert (R–IL), whose district includes Argonne, "often because doing so is in the best interests of the nation for other reasons." By giving DOE its marching orders, Congress has revived the debate over exactly what those interests are.

–ELI KINTISCH

SCIENTIFIC PUBLISHING

NIEHS Journal Is on the Block

The new director of the National Institutes of Health's (NIH's) environmental institute has drawn flak by proposing to sell off the institute's well-regarded journal.

In September, David Schwartz requested public comments on privatizing the journal as part of an "ongoing review" of programs. Dozens of scientists and environmental and health groups have reacted in horror, fearing the loss of the journal's mix of research and news, now free online. Some also worry that a commercial owner would be less likely to publish findings unflattering to industry. Last month, a dozen Democratic members of Congress chimed in, writing NIH Director Elias Zerhouni that privatizing the journal "places at risk the integrity and quality" of *Environmental Health Perspectives* (*EHP*).

The 33-year-old *EHP* is published by the National Institute of Environmental Health Sciences (NIEHS), a branch of NIH in Research Triangle Park, North Carolina. It publishes original research and news in a subscription-based print edition and free online. *EHP*'s impact factor (a measure of how often its articles are cited) of 3.93 ranks it second in environmental science behind *Global Change Biology*. *EHP* Editor-in-Chief Thomas Goehl says the journal's $3.3 million annual budget supports the news section, a student edition, and translations of summaries for developing countries as well as the publication of peer-reviewed research.

Hands off. Many scientists want NIEHS to keep its journal.

Since the institute announced its proposal in the 19 September *Federal Register*, more than 70 mostly academic researchers—including members of *EHP*'s editorial board—have signed a letter voicing "strong opposition" to the move. They fear that nobody else will want to publish its mix of toxicology, epidemiology, medicine, and risk analysis, that developing countries would lose free access, and that *EHP*'s "extras" such as news coverage of "complex science" would be discontinued. Some scientists also worry about *EHP*'s independence. "A commercial publisher may be less willing to publish articles that have implications for powerful interests," suggests epidemiologist David Michaels of George Washington University in Washington, D.C.

Some environmentalists worry that privatizing the journal could be part of what they perceive as a shift away from examining the risks of pollutants and toward studying clinical disease. "The E in NIEHS is going silent," claims toxicologist Jennifer Sass of the Natural Resources Defense Council in New York City.

Schwartz declined to be interviewed, but NIEHS noted in a statement that the government publishes few scientific journals. (In 1997, for example, the only other major NIH-published journal, the *Journal of the National Cancer Institute*, was spun off and is now published by Oxford Press.) NIEHS also argues that maintaining *EHP* as a government publication "may actually limit the journal's independence and potential future growth." The institute expects to make a decision in the next few months.

–JOCELYN KAISER

U.S. GRADUATE EDUCATION

Universities Must Pay to Play in Ph.D. Program Rankings

How does your doctoral program stack up against the competition? It may cost your university $20,000 to find out.

The National Academies' decadal assessment of more than 4000 research doctoral programs at 300-plus U.S. universities is a must-read for both elite schools and those hoping to move up in the pecking order (*Science*, 23 June 1995, p. 1693). Begun in 1981 and repeated in 1993, it's a massive compendium of information for anyone interested in understanding a system acknowledged to be the best in the world. It also features a reputational ranking that allows universities to claim bragging rights—or to find out how much they need to improve (*Science*, 12 December 2003, p. 1883).

Such a gargantuan effort doesn't come cheap. Academy officials say that a tight budget severely limited their ability to analyze much of the data collected from the 1995 survey, and an outside panel offered several suggestions for improving the process. So the academy quadrupled the budget for the next go-round (which will consist of four online questionnaires to administrators, departments, faculty, and graduate students in five fields), and study director Charlotte Kuh took on the task of raising the $5.2 million needed.

Two foundations quickly chipped in $1.2 million, but the National Science Foundation (NSF), which has pledged $400,000, and the National Institutes of Health, which is ponying up $550,000, said the funding was contingent on the universities themselves anteing up. "They have more at stake than the government does," explains NSF's Nathaniel Pitts, "and more interest in the departmental rankings."

Given those marching orders, Kuh developed a sliding scale based on the size of institutions' graduate programs. Schools that produce 100 or more Ph.D.s a year will be assessed $20,000; those graduating between 50 and 100 students will pay $10,000; and the smallest institutions will be charged $5000. The fee is mandatory, she adds: "We can't afford any free riders." Those fees will generate $2.1 million, Kuh estimates, adding that she has a line on the rest of what's needed.

John Vaughn of the 62-member Association of American Universities, which represents most of the country's top research institutions, says he hasn't heard of any universities that plan to opt out, and Kuh says an initial mailing last month to 160 of the largest schools has already yielded 50 pledges. But it's a grudging acceptance. Graduate school deans feel they are already financing the project by paying for the people and resources needed to collect the data, Vaughn says. "We're also concerned about the possible precedent it sets," he adds. "What if every federal program that benefits universities were to ask us to contribute?"

Assuming all goes well, Kuh hopes to post the first questionnaire in April and to offer a summary of all results by the end of 2007. "It will provide more information, derived from a more objective process" than previous surveys, promises Jeremiah Ostriker, a professor of astrophysics at Princeton University and chair of the committee overseeing the study. "I think it'll be a remarkably useful effort."

–JEFFREY MERVIS

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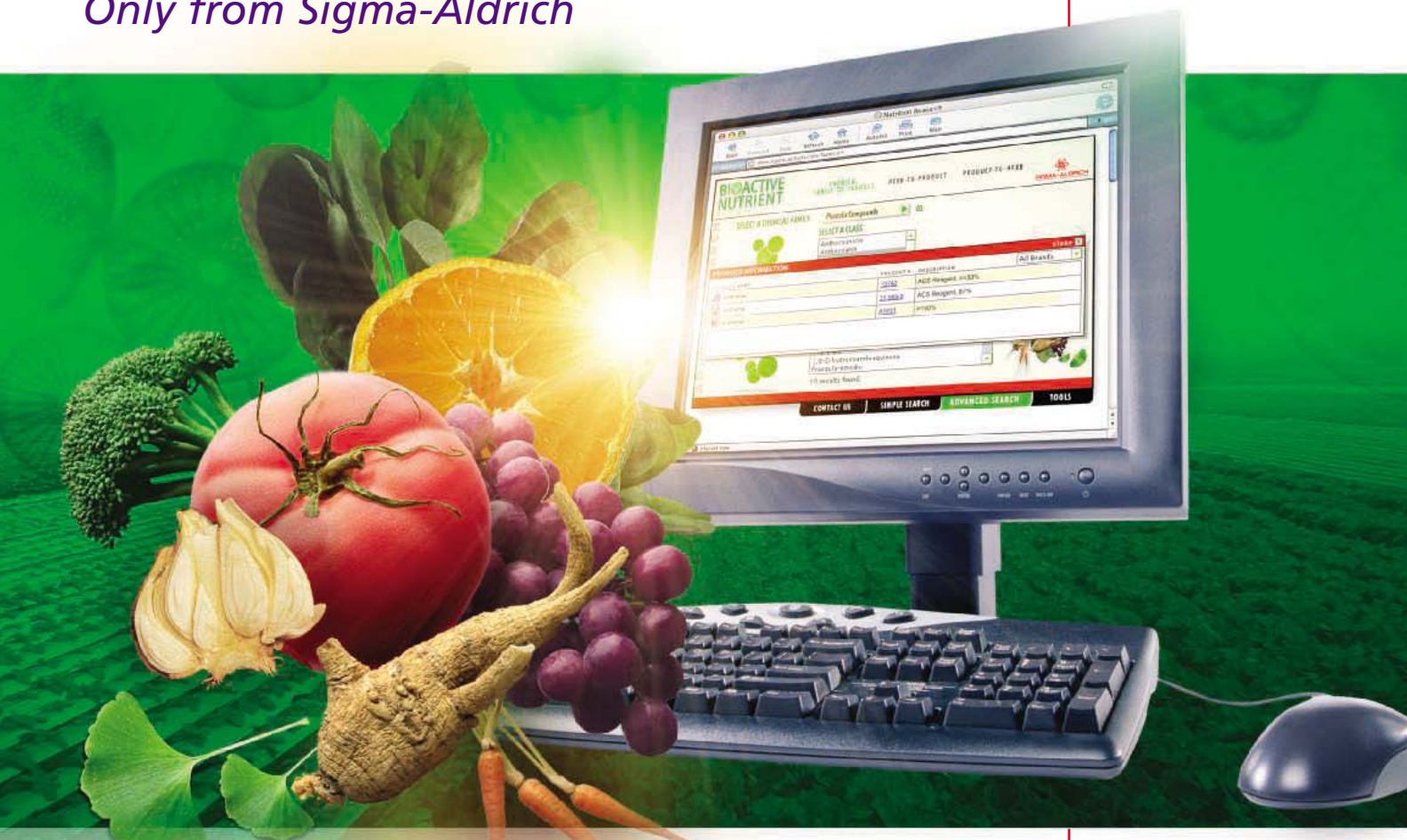

Designed to help you locate the chemicals and kits needed to support your work, the **Bioactive Nutrient Explorer** is an Internet-based tool created to aid medical researchers, pharmacologists, nutrition and animal scientists, and analytical chemists studying dietary plants and supplements. The **Bioactive Nutrient Explorer** identifies the compounds found in a specific plant and arranges them by chemical family and class. You can also search for compounds having a similar chemical structure or for plants containing a specific compound. Convenient resources include an **Herb Finder** for common/Latin name synonyms of a given plant, as well as a **Product/Class Finder** that identifies the chemical classification of a compound and takes you to a list of related compounds. When you've found the product you need, a simple mouse click links you to our easy online ordering system.

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SPACE SCIENCE

Fuel Shortage Imperils Asteroid-Sampling Mission

Japanese scientists are confident that the tiny spacecraft Hayabusa picked up rock fragments it blasted from the surface of the near-Earth asteroid Itokawa during a brief touchdown on 26 November. Unfortunately, the craft might never deliver its cargo. At press time, ground controllers were having trouble communicating with Hayabusa and feared its rockets might be out of fuel. But images and data already transmitted back to Earth could overturn current understandings of asteroids, says Akira Fujiwara, mission chief scientist for the Institute of Space and Astronautical Science, part of the Japan Aerospace Exploration Agency.

If the mission succeeds, the fragments will be the first returned from a planetary body since Apollo astronauts hauled back their last load of moon rocks almost 35 years ago. Derek Sears, a professor of space and planetary sciences at the University of Arkansas, Fayetteville, predicts that Hayabusa's "technically astounding achievement" will inspire other sample-retrieval missions to asteroids, comets, and perhaps even Mars. "This shows how it can be done and for a reasonable cost," he says.

Hayabusa has already overcome daunting setbacks. En route to Itokawa, the craft lost two of three gyroscopelike reaction wheels that control attitude. To compensate, team members fired small rockets originally intended for course corrections. Jun'ichiro Kawaguchi, Hayabusa project manager, says the rockets enabled them to orient Hayabusa but tended to push the craft off course.

Pyrrhic victory? Rocket problems might keep Hayabusa from returning its samples to Earth.

Kawaguchi says the difficulties of using the rockets likely contributed to mishaps earlier this month, when the team abruptly aborted a rehearsal descent and accidentally released a small rover into space instead of onto Itokawa. But the scientists learned from their mistakes and made a successful touchdown on 20 November and retrieved the sample 6 days later.

The biggest challenge lies ahead. Hayabusa is short of fuel for the rockets used for course corrections; team members will have a limited ability to keep Hayabusa's ion engine on course during the 300-million-kilometer return journey.

Meanwhile, planetary scientists are busy analyzing more than 1500 images Hayabusa transmitted back to Earth, along with data from infrared and x-ray spectrometers and a laser altimeter. Mission chief scientist Akira Fujiwara says they are already seeing surprises. Itokawa is strikingly different from the asteroid Eros, which NASA's NEAR Shoemaker spacecraft visited in 2001. For starters, the surface of Eros was covered by a regolith of powdery debris created by weathering and meteorite collisions. The surface of the much smaller Itokawa is bare rock. Fujiwara says Itokawa's weak gravitational pull "makes it very difficult to accumulate anything on the surface." That means the space-weathering process could be dramatically different for asteroids of different sizes. Fujiwara promises that publications will start appearing within months.

–DENNIS NORMILE

EPIDEMIOLOGY

Talk on 'Underground' Bird Flu Deaths Rattles Experts

A senior Japanese virologist and adviser to the World Health Organization (WHO) roiled the influenza field last week when he suggested—during what he believed was a private gathering in Germany—that China had concealed hundreds of human bird flu deaths. That's how several people, including two reporters, interpreted a talk by Masato Tashiro, director of the National Institute of Infectious Diseases in Tokyo. But Tashiro denies that he made any such allegation, saying he only meant to say that surveillance in China is poor.

According to the *Frankfurter Allgemeine Zeitung* (*FAZ*) newspaper, Tashiro stunned an audience on 18 November that had gathered to mark the retirement of University of Marburg virologist Hans-Dieter Klenk. *FAZ* reported that Tashiro showed a table documenting several dozen outbreaks of the bird flu strain H5N1 in China, whose toll included at least 300 human deaths, seven cases of probable human-to-human transmission, and more than 3000 people in quarantine. "We are systematically being deceived," the story quoted Tashiro as saying. Official records list only three confirmed human cases of H5N1 in China, two of them fatal. Given the number of avian outbreaks, many virologists wonder why China hasn't seen more human cases, says virologist Peter Palese of Mount Sinai School of Medicine in New York City.

Tashiro's allegations appeared on ProMED, an e-mail list, on 23 November. China's foreign ministry quickly denounced them as "baseless."

In an interview with *Science*, Tashiro called the *FAZ* story "misleading." He did show a table with information given to him by "a friend" in China, he says, but just to illustrate the type of "underground rumors" currently circulating. "The friend is reliable, but the information itself, I don't know," says Tashiro, who says his only point was that China may be missing H5N1 cases. "I don't think they are concealing any important facts."

But several people at the meeting took away a different message. The German radio network WDR carried a story similar to *FAZ*'s, reporting "more than 200" deaths. "We were all flabbergasted; we didn't want to believe it," says virologist and biochemist Michael Schmidt of the Freie Universität in Berlin, who was in the audience. "Tashiro is deeply convinced there have been at least 200 deaths; … he's very concerned. Maybe he thought this was just a small circle of friends where he could say a little bit more." Klenk also says the *FAZ* story was a "correct" reflection of the talk.

China's relations with WHO have been rocky since the country was caught hiding the true extent of the SARS epidemic in 2003. But China is cooperating well on bird flu, says Klaus Stöhr of WHO's influenza program. And although many people think the Chinese government may be missing human H5N1 cases, WHO has no reason to believe that it is concealing them, Stöhr says.

–MARTIN ENSERINK

With reporting by Gong Yidong in Beijing.

ILLUSTRATION: A. IKESHITA/MEF/ISAS

To make islands safe for rare native species, biologists are mounting increasingly complex campaigns to shoot, trap, or poison exotics

Winning the War Against Island Invaders

Santa Cruz Island, California—It is the coldest, blackest hour before dawn, and Norm MacDonald's professional killers are getting ready. In the doorway of a map-filled war room, Ace is cleaning the sight on his .223-caliber rifle and working the bolt. Steve, sipping tea, straps on a pouch of hollow-point ammo good for blowing baseball-size holes in flesh. Then they step outside to the helicopter that will take them to the enemy: 5000 feral pigs roaming this 250-square-kilometer landmass. "The boys," as MacDonald calls his team in his soft-as-rain New Zealand accent, "are not just hunting. This is eradication."

Every day around the world, terminators are pursuing human-introduced creatures accused of threatening island biota, and, increasingly, wiping out every last invader. It's just a dream on the mainland, where exotic invaders such as nutria or zebra mussels can only be controlled, because once a patch of woodland or water is cleared there are always more in the next. But on islands, humans have proven good at finishing the job because space is limited and the exits sealed: Consider the dodo.

Scientists have focused their attention on islands because they are among the richest and most vulnerable of the world's ecosystems. They cover 3% of Earth but house 45% of bird, plant, and reptile species. Introduced species are endangering many of the natives, because many island creatures are endemic. They have not evolved defenses against the mainland predators and grazers that humans bring—rats, cats, sheep, goats, and pigs. Islanders often get outcompeted or eaten; biologist Bernie Tershy, director of Island Conservation, a California-based nonprofit that specializes in eradications, says that since 1600, islands have accounted for up to 90% of bird and reptile extinctions worldwide, and half those of plants and mammals. Rats, now on 80% of islands, attack plants, insects, birds, and small animals; they are implicated in about half of recorded bird and reptile extinctions. Goats eat whole trees and gnaw plants to bare rock. On Hawaii's remote Laysan, rabbits eliminated 26 plant species within 20 years after arriving in the 1900s. On the Indian Ocean's subantarctic Kerguelen Archipelago, one cat and her three kittens arrived in the 1950s, and by the 1980s, they had reproduced into 3500 felines killing 1.2 million seabirds a year.

Ecologists once thought it impossible to wipe out invaders, even on islands. Into the 1980s, "hardly anyone thought eradication could be done," says Daniel Simberloff, an ecologist at the University of Tennessee, Knoxville, who was an early advocate. But efforts on hundreds of islands worldwide have proven that mammals, at least, can be taken out, although campaigns against plants, insects, and reptiles are much tougher. Now exterminations in the name of conservation are taking place on ever-bigger islands, with ever more military-style planning and hardware. The key, say experts, is to attack fast and get every last individual before they can reproduce, adapt, or escape, because even a few strays can quickly rebound.

New studies show that some threatened species recover spectacularly. "The problems are obvious, and the solutions are obvious," says Tershy. However, this "nasty necessity," as Tershy calls it, is not always simple. Subtracting one invader from an ecosystem can make other components run amok, and the slaughter cannot always bring back rare native species to environments that have been severely altered. Then there is human ecology, as animal-rights protesters increasingly try to thwart extermination efforts. Together, these complications can weave a plot as tangled as a history of the Hundred Years' War. Santa Cruz is Exhibit A.

Coming back. Without rats, Xantus's murrelet chicks are rebounding on Anacapa Island.

Extermination island

About 100 kilometers northwest of Los Angeles, Santa Cruz is the largest of the 12 Channel Islands of California and Mexico. Their precipitous canyons, woodlands, and prairies hold some 2000 species and subspecies. Approximately 140 are endemic to one or a few islands, including the gigantic island scrub jay; the island spotted skunk (said to smell nicer than mainland cousins); dozens of flowering plants; and six subspecies of cat-size foxes, each peculiar to its own island. Some 10,000 years ago, Chumash people arrived with imports such as oaks; after 1800, Europeans brought smallpox, pigs, sheep, garden plants, and honeybees. The Chumash disappeared, alien grasses spread to 75% of Santa Cruz, and by the early 1900s, creatures such as the island sparrow and the Santa Cruz monkey flower were extinct.

Attempts at control came as early as 1904 after livestock escaped and started denuding the land. Hunters shot tens of thousands of sheep and pigs. But they never got them all. As soil eroded and nearly a dozen plants approached extinction in the

CREDITS (TOP TO BOTTOM): SEAN MCKNIGHT/COURTESY OF THE NATURE CONSERVANCY; NATIONAL PARK SERVICE

1980s and 1990s, The Nature Conservancy (TNC) and National Park Service (NPS) took over Santa Cruz and other islands and got serious.

Research on invasives was just taking off, but most scientists were at first focused on documenting invaders' effects, not designing ways to kill them off. New Zealand's Department of Conservation (DOC) proved the worth of counterattack. Officials enlisted the country's deep-rooted hunting culture to attack large mammals, which are fewest, most visible, and usually the most destructive introduced creatures. Starting with islands of a few acres, New Zealanders hunted and trapped deer, goats, and pigs. They fenced islands into sectors, corralled animals in traps, ambushed them from helicopters, and made skirmish-line ground sweeps with tracking dogs. Eradication became an industry, and Norm MacDonald, a hunter since childhood, started his outfit, Prohunt Ltd. "We try to stay humane," says the teddy-bearish CEO, an expert at shooting from a helicopter. "One shot for the head, one for the body—it's all over really quickly."

As New Zealanders added poisons and traps to the arsenal and moved to smaller prey such as possums and rabbits, the idea caught on. In the past 8 years, Island Conservation has rid 27 Mexican Pacific islands of 41 mammal populations, including hard-to-catch cats. Scientists rarely do the killing themselves, though. "The last thing you want is a bunch of biologists running around with guns," says Josh Donlan, a conservation biologist at Cornell University. Island Conservation's muscle is longtime Oregon fur trapper Bill Wood. "I learned from the old guys," says Wood, co-author of two chapters in the 2002 book *Turning the Tide*, a collection of island-eradication papers. For cats, Wood relies on night shooting with spotlights—a poacher's favorite—and elaborately engineered traps. Rabbits rarely escape Wood's Jack Russell Terrier, Freckles, who finds their burrows and digs them out.

By the 1990s, DOC had shown that even rats can be wiped out, with poison pellets dropped from helicopters. In 2006, the agency hopes to confirm the world's largest rat eradication: 11,300-hectare subantarctic Campbell Island, hit in 2001 with 120 tons of the poison brodifacoum. Seabirds decimated by the rats are coming back fast. To protect nontarget animals, including rare seabirds, teams here and elsewhere tint pellets in bright colors that nontarget species reject, use bait stations they can't get into, or remove them to captivity until baits decay. Toxins deadly only to rats and cats are also in the works.

Most of these strategies are in use in the world's largest eradication project, on Ecuador's Galápagos Islands, funded by $21 million from the United Nations and private foundations (*Science*, 27 July 2001, p. 590). The flagship target is 150,000 goats on 458,000-hectare Isabela Island. Project leader Felipe Cruz says that 90% of the job is killing the last, canny survivors; a few goats can elude hunters for years, nimbly roaming over near-impossible terrain and hiding at the sound of a helicopter. So the project employs new methods, including deployment of 600 radio-collared, companion-seeking "Judas goats" that lead helicopter-borne shooters to holdouts. The group also has sterilized "Super Judas" nannies, implanted with hormones to draw billies. To assure total coverage, aircraft, ground hunters and even dogs are fitted with Global Positioning System units that record their movements, all integrated daily into a Geographical Information System. Final mop-up may involve airborne forward-looking infrared radar to generate thermal images of animals hidden in underbrush, the system used by U.S. Special Forces to hunt guerrillas. Cruz won't say how many goats they have killed so far, but he thinks it will all be over by March 2006. The work is described in the October issue of *Conservation Biology*, and papers are in press at *Wildlife Research* and *Applied Animal Behavior Science*.

Goat attack. Invasive goats, such as these on Isabela Island in the Galápagos, can strip vegetation completely.

Judas pig. Hunters put a radio-transmitting collar on a pig so it can lead them to other pigs.

In the Channel Islands, many eradications have preceded the war against the pigs, including removals of rabbits, cats, burros, horses, and cattle. TNC had all 37,171 sheep shot from its portion of Santa Cruz by 1987, and NPS, which owns the rest, deported 2000 remainders alive in 2001.

When introduced species are gone, native creatures often bounce back dramatically. NPS reports that with livestock removal, riparian plants on modest Santa Rosa Island have gone from virtually zero in 1995 to 90% coverage today. Since NPS poisoned rats on tiny Anacapa Island in 2001 and 2002, nesting by rare Xantus's murrelets has increased 80%. Further south, on Mexico's Guadalupe Island, a half-dozen species of plants long thought extinct have suddenly reappeared in the last year or so, along with

CREDITS (TOP TO BOTTOM): JOSH DONLAN; SEAN MCKNIGHT/COURTESY OF THE NATURE CONSERVANCY

150-some seedlings of nearly extinct endemic Guadalupe pines, even as Island Conservation mops up the last of 7500 goats there. In Alaska's Aleutian Islands, rare seabirds such as fork-tailed petrels have increased four- to fivefold within 10 years of fox removals, says Vernon Byrd, a biologist with the Alaska Maritime National Wildlife Refuge. And in New Zealand, biologists cite dozens of native invertebrates, reptiles, and birds that have rebounded after eradications (68 of the nation's 168 mammal-invaded islands are now cleared). On Korapuki Island, one rare skink increased 30-fold when rats were taken off, according to a review just published in *Biological Invasions*.

Biologists are in fact so convinced of these successes that many do not bother doing extensive studies on the results, writing them up only in conference proceedings or internal reports. "You don't need a guy with a Ph.D. and 10 years of data to tell you the obvious: An insect that was practically nonexistent is now everywhere. Most people put the resources into doing the next job, not proving they did the last one," says C. Richard Veitch, an ex-DOC biologist now with the World Conservation Union. He may be right in some cases, says Cornell's Donlan, but scientists are realizing that as they move to larger, more complex islands with multiple invaders, they need long-term peer-reviewed follow-up, because results can be confusing.

On Santa Cruz, some ecologists think that earlier eradications may actually have helped make the war on pigs necessary. Removing sheep might have helped pigs overmultiply by giving them more forage and cover, says wildlife ecologist Bruce Coblentz of Oregon State University, Corvallis. The pigs till soil to 30 cm or more, making much of the island a lumpy mess, endangering nine species of endemic plants, and preventing gnarly old oaks from having descendants. (Pigs love acorns.)

Worse, says Gary Roemer, an ecologist at New Mexico State University in Las Cruces, it appears that by about 1994, tasty piglets had attracted mainland golden eagles. They may also have come earlier for the tens of thousands of sheep carcasses that TNC left scattered, notes University of California (UC), Santa Cruz, field biologist Brian Latta. (The goldens previously were kept out by fierce fish- and carrion-eating bald eagles, but the balds were wiped out in the 1960s by DDT.) Roemer says the golden eagles then discovered a convenient food source in the tiny, unwary Santa Cruz Island foxes, which plummeted from an estimated 1500 in 1994 to 150 today. Nearby islands' foxes nearly disappeared too, and in 2004, three subspecies were declared endangered. TNC biologists say that as long as the fast-reproducing pigs are on Santa Cruz, they will provide abundant food for the goldens, which will stick around and eventually extinguish the slow-reproducing foxes. Scientists agree: The pigs must go.

The last pig

Killing the pigs and cleaning up the ecological mess after them is a huge operation. MacDonald and the boys have been living in an isolated old ranch house since this summer, after Prohunt was selected for the $6.2 million, 3-year project. TNC has fenced its land into five kill zones to keep fugitives contained. At the same time, there are programs to live-trap and relocate golden eagles to the mainland, reintroduce young bald eagles, and breed foxes in captivity.

It is now morning. After dogs and men take off in the copter, MacDonald and TNC official Julie Benson drive to a high hilltop in Zone 2 to watch them disembark. As of today, they have killed 2574 pigs. Except for a few used for pig roasts for the boys, the team piles up the dead in remote spots and covers them to keep off scavengers—and the eyes of visitors, who TNC officials feared may turn against the project if they see the results. Yesterday, MacDonald's crew was pursuing what appear to be the last four pigs in this zone, but they got only three. "Not good; now that pig is educated," said MacDonald. "Every time we see an animal, we try to make sure that's the last time it sees us. If you know what I mean." But as experience suggests, the last pig is not necessarily the end of the story.

After TNC shot the Santa Cruz sheep and removed cattle, 33 of 43 endemic plant species came back, including the endangered Santa Cruz silver lotus and northern island *Hazardia*, spreading outward from cliffside refuges to which the livestock had pushed them. However, for unknown reasons several native plants have actually declined. More significantly, livestock are no longer eating exotic plants either, says Steven Junak, a botanist at the Santa Barbara Botanic Garden. As a result, previously invisible bamboolike fennel, a sheep and cattle favorite, has carpeted 5% of the island, higher than a man's head, perhaps spread in part by pigs. TNC has sprayed the fennel with herbicide, but it only gets replaced by fast-moving alien grasses, according to a recent paper in *Biological Conservation*. Star thistle and hundreds of other plants are coming in elsewhere, defying volunteer crews who pull them up by hand.

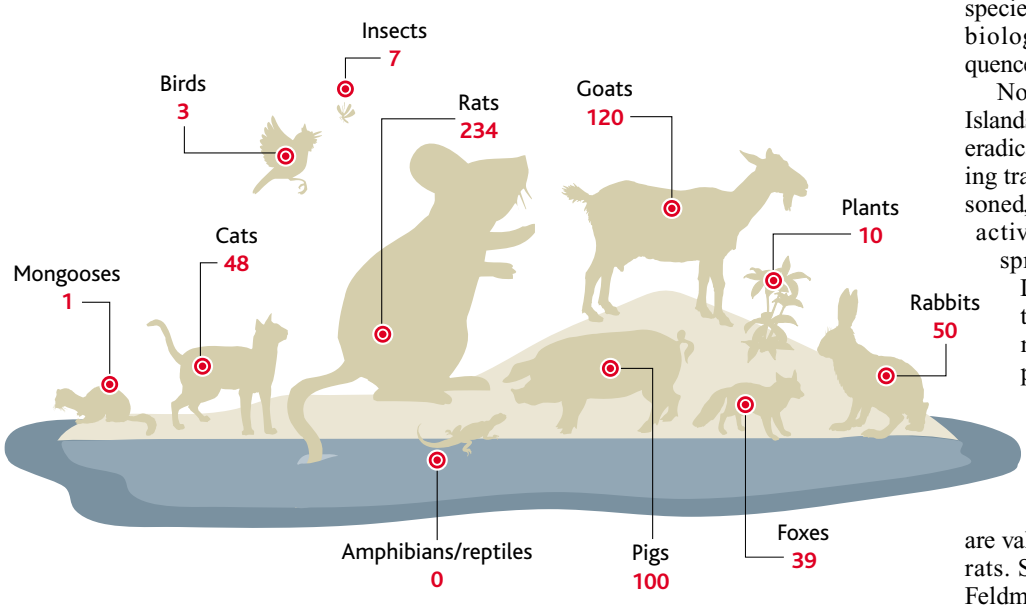
This is especially troubling because plants are nearly impossible to eradicate once they get going, says botanist Marcel Rejmanek of UC Davis. He says infestations of a hectare or less can usually be wiped out, but it takes up to 10 years; those occupying 1 to 1000 hectares might be done in 30 years; and anything bigger is impossible. "Early detection is the only hope; once something is a problem, it's too late," he says. (Reptiles such as the brown tree snake, which has extinguished practically all native birds, lizards, and bats on the Pacific island of Guam, seem

Before and after. Except for an exclosure area, the removal of goats and pigs transformed the highlands of Santiago Island (above) in the Galápagos.

CREDITS (BOTH): PROJECT ISABELA, GNP & CDF

ERADICATION SCORECARD

Number of islands where documented eradications have finished off . . .



CREDIT: K. BUCKHEIT/*SCIENCE*

equally resistant, along with insects, which have been removed from only a half-dozen islands worldwide.)

Santa Cruz is not the only place to suffer ecological kickback. After goats were removed in 1998 from the Pacific's Sarigan Island, native forests sprouted fast—along with *Operculina ventricosa*, an exotic vine that no one even knew was present until the goats stopped eating it. It has now developed an uninterrupted carpet over parts of the island. Similar plant invasions have occurred on other islands after removal of pigs and rabbits. On various subantarctic islands, cats have been wiped out, leading to rat expansion; rabbits have been killed, expanding exotic grasses that in turn become habitat for rats; exotic possums and rats have been exterminated, but not possum- and rat-eating stoats, which then switch to eating native bird eggs; and so on. In 1979, Amami Island, Japan, imported 30 mongooses to control rats and poisonous snakes, but the mongooses instead ate crops and rare endemic birds, and they have now multiplied out of control, too wily to eradicate.

"There are many instances where common sense tells you that cleaning up one mess may create a second mess, but some things are predictable only in hindsight," says ecologist Erika Zavaleta of UC Santa Barbara. David Wardle, an ecologist at Landcare Research, says long-term predictions are hard to make, because invaders may alter the very chemistry and microbiology of island soils. Out-of-whack ecosystems may reestablish a balance, he says, but it might take hundreds, even thousands, of years. Eradication is only a first step, he says; soil amendments, planting of native flora, and animal reintroductions often must follow. "We should forget the word 'restoration,' " says UC Davis wildlife biologist Rob Klinger, who has worked on Santa Cruz. "You can never put things back exactly as they were."

It is now late morning, and the boys are heading back to the ranch house in a pickup over a rough dirt track. On the flatbed, a fresh, bloody pig jaw with two sharp tusks jumps up with each big bump—unfortunately, a souvenir from yesterday, not today. That last pig in Zone 2 got away again. "Don't worry," says Steve. "We'll get him."

After that job is done, there may be other challenges, including the golden eagles. It has taken 6 years and close to $1 million to capture and relocate just 42 with traps. About six holdouts are left—the wariest, says Brian Latta, who led the project until February. Now, with the pigs disappearing, he, Roemer, and others fear that the birds could attack the foxes even harder and finish them off in the wild. Golden eagles are common, so Roemer and others agree that the solution is easy: Shoot them, too (*Science*, 28 November 2003, p. 1532). But eradicating native wildlife—an American icon no less—"is *very* politically incorrect," says TNC's Benson. TNC and NPS will keep trying to catch the goldens alive, even if it slows the foxes' recovery, she admits. Meanwhile, exotic wild turkeys are on the horizon. They are common on Santa Cruz, and the pigs probably eat their eggs, so biologists fear that once the pigs are gone, the turkeys could become a plague as they are on the mainland, where they vacuum-clean forest floors of amphibians and insects.

All this may seem like a never-ending saga of manipulation, but against the grim backdrop of worldwide extinctions, eradications are worth it, say conservation biologists; they are one of the few clear success stories. "We're dealing with highly threatened species," says Keith Broome, a senior DOC biologist. "We already know the consequences of doing nothing."

Not everyone agrees. On other Channel Islands, animal-rights protesters have slowed eradications by cutting fences and vandalizing traps. When the Anacapa rats were poisoned, the Fund for Animals sued, and two activists were arrested after landing to spread rat feed laced with an antidote. Local activists are currently suing over the pigs and flying "save the pigs" banners across the skies. They say that if pig numbers must be reduced, it should be done by means of contraception or relocation. TNC counters that neither of those methods will get every pig.

Protesters say all individual animals are valuable, whether rare foxes or common rats. Santa Barbara businessman Richard Feldman, a co-plaintiff in the pig suit, says scientists have "demonized" the pigs and that evidence linking them to fox declines is thin or fabricated. He adds that pigs are now part of the island, such as oaks and foxes, which also once came from elsewhere. "Ecosystems are always changing. Scientists want to play god," he says.

Conservation biologists tend to side with endangered species. "Animal rightists are a bunch of well-meaning pinheads who just don't understand," retorts Coblentz. However, some biologists agree that deifying science is a mistake. "We shouldn't confuse scientific knowledge with moral authority. Observing extinction and deciding what to do about it are different, and there all human beings have a valid point of view," says Dov Sax, a research biologist at UC Santa Barbara. In a recent essay in *Austral Ecology*, Sax and ecologist James Brown of the University of New Mexico, Albuquerque, call for scientists to take more care in studying how species interact before deciding which ones to declare war on. Many "alien" species are not harmful to natives, points out Sax, and simply become part of the mix. "It is not to suggest that modern humans should … elect not to intervene," they say. "It is to plead for more scientific objectivity and less emotional xenophobia."

On Santa Cruz, the story is still in progress. By midafternoon, Julie Benson is bumping along a dirt road in a Land Cruiser heading for the mainland ferry. Suddenly, a flash of hair and legs flits from the scrub on the left side and disappears in the scrub on the right side. It is a black-and-white adult pig, trailed by a baby. Both are running as fast as they can.

–KEVIN KRAJICK

Kevin Krajick is the author of *Barren Lands: An Epic Search for Diamonds in the North American Arctic*.

String Theory Meets Practice as Violinmakers Rethink Their Craft

Incorporating innovative designs and novel materials, bright and responsive "ultralight" instruments may be the sound wave of the future

KING OF PRUSSIA, PENNSYLVANIA—A little thin down low, the sound of the violin blossoms as Bach's unaccompanied sonata in C major wends into the upper registers. Close your eyes, and you can almost see the instrument making the bright, crystalline sound, its classic form curving as gracefully as the music, its amber finish enriched with nicks and scrapes accumulated over the centuries, its compact body resonating with the very emotion of the soloist. It may be best to keep your eyes closed, however.

In fact, the instrument looks less like a violin than a model airplane gone horribly wrong, and it's hard to reconcile the beauty of the sound with the device's homely appearance. A latticework of spars covers its asymmetrical balsawood body. Crude vents perforate its top where a traditional violin's elegant "f-holes" would lie. Yet the thing sings to the violinist's touch. "The sound is just enormous under the ear," says Annalee Patipatanakoon of the Gryphon Trio, a chamber group based in Toronto, Canada. "Wow!"

Innovator. Joseph Curtin is one of a small group of violinmakers experimenting with new designs and materials.

The odd contraption exemplifies the innovative approach some violinmakers are taking to the hallowed instrument. For decades, scientists have tried to explain the violin's captivating sound and the supposed superiority of instruments made 300 years ago by Italian masters such as Antonio Stradivari and Giuseppe Guarneri. Now, a handful of top makers are embracing scientific methods and striving to move beyond copying the "old Italians." Several have gathered here to report their progress to the Violin Society of America (VSA)* and encourage others to follow their controversial lead.

"I've been trying to step outside and say, 'Hey, is [the traditional design] perfect?' " says Joseph Curtin, a violinmaker from Ann Arbor, Michigan. "In some ways it may be, but the more I look into the design, the more it looks rife with things that could be improved." Such efforts have begun to attract attention outside le métier. In September, the Chicago, Illinois–based John D. and Catherine T. MacArthur Foundation awarded Curtin a $500,000 "genius grant" for his use of acoustic science, innovative designs, and novel materials such as balsa wood and carbon-fiber composites.

Some aficionados say the traditional wooden violin could use a rethink. "We are at the beginning of a revolution," says Fan-Chia Tao, an acoustical engineer with string manufacturer J. D'Addario & Co. in Farmingdale, New York. "Within a generation, the wooden violin will be as obsolete as the wooden tennis racket or the wooden golf club." But others hesitate to fiddle with the fiddle. "I think that many who engage in [the scientific approach] feel that they'll be able to make Stradivariuses like you make Ford Explorers," says Hans Tausig, former president of VSA, from his home in Forest Hills, New York. "And that's where they go wrong."

Sonic lighthouse

A work of art, a historic artifact, a million-dollar investment: A fine old violin is many things. But when it comes to making music, a violin is a tool for producing sound. A violinist sets a string vibrating by bowing it and fixes the frequency, or pitch, of the vibration by pinning the string against the fingerboard. The string pushes the bridge, a wooden stanchion that suspends the strings above the top of the instrument, and the jiggling bridge forces the body of the violin to vibrate, too. The moving body pushes the air to create sound.

That seems simple enough, but the character of a violin emerges from the subtle details. The vibrating body can contort in many distinct patterns of motion, or "modes," depending on the frequency. For example, at frequencies around 285 cycles per second, the top and bottom of the body move in opposite directions, as air flows in and out through the f-holes in the top. Thanks to the myriad overlapping modes, a violin cranks out certain frequencies more efficiently than others, and the differences give the instrument its distinctive voice.

The violin also acts like a sonic lighthouse, beaming its sound in specific directions, explains Gabriel Weinreich, a physicist retired from the University of Michigan, Ann Arbor. The directions change rapidly as the frequency changes, so that even the slightest wiggle of the player's hand—such as the shaking "vibrato" violinists use to embellish notes—causes the direction of the sound to vary dramatically. Known as directional tone color, that phenomenon may explain why a good violin sounds "alive," Weinreich says.

And all agree that the best old Italians possess a buttery, lively sound that has set the standard for violins for centuries. Through innovations of their own, the Italian masters of the late 17th and early 18th centuries developed a design that violinmakers have copied religiously ever since, sometimes down to the blemishes in the finish. But a few makers are trying to push past the bounds of tradition.

For ages, people have tinkered with the violin. In the 1970s, aeronautical engineer Leonard John, currently with Bombardier Aerospace in Downsview, Canada, developed a carbon-fiber violin. And the grand dame of violin research, Carleen Hutchins of Wolfeboro, New Hampshire, produced a variety of novel instruments and inspired Curtin and others. But now, makers with sterling reputations for producing top-quality traditional instruments are embracing the insights of science, says Jeffery Holmes, a violin restorer and dealer in Ann Arbor. "They're interested in how the violin works and how science applies to it," Holmes says.

CREDIT: A. CHO/*SCIENCE*

* 33rd Annual Convention, 10–13 November 2005.

Mapping modes, sculpting sound

At the least, a scientific approach should help produce instruments that sound more like the old Italians. Martin Schleske, a maker in Munich, Germany, has mapped the modes of classic instruments and analyzed the sound they radiate when tapped on the bridge, measuring the relative strengths of the constituent frequencies. He uses the data to make "tonal copies" that mimic the voice of the originals. "A lot of musicians say it's great," Schleske says in a phone interview, "because there is now a way of getting an objective measure of an instrument."

Taking a different, rather irreverent tack, Samuel Zygmuntowicz, a maker from Brooklyn, New York, is experimenting with tailoring the sound of an inexpensive violin by simply gluing small strips of wood to it. The spars stiffen the instrument and alter its modes. "I started as a sculptor," Zygmuntowicz says, "and to me what's exciting about this is I can shape sound the way I used to shape clay." Such experimenting could help pinpoint the origins of a fine violin's superior tone.

But innovators are striving not merely to produce a better knockoff of a Stradivarius but rather to achieve something new. In particular, they argue that violinists will always opt for instruments that project more sound and respond more quickly. Makers might produce them by using materials as stiff as, but lighter than, the spruce traditionally used for violin tops and backs, says Norman Pickering, an acoustical engineer in East Hampton, New York, and a consultant to D'Addario. For a given amount of energy, the lighter stuff will move more and create a louder sound. Also, because the material has less inertia, the instrument should switch from note to note more readily, provided that the friction within the material, or "damping," is about right.

So violinmakers are experimenting with light, stiff materials such as carbon fiber and balsa wood. "When you get a lot lighter than traditional [materials], you get an immediacy of response that's almost shocking," Curtin says. He has brought to the meeting a violin whose vacuum-molded top and back consist of two plies of balsa covered with a thin laminate of spruce. Stripped of the corners and curlicues that adorn a traditional violin, the instrument looks at once old and modern, its economical lines hearkening back to the architectural designs of Frank Lloyd Wright. It sings sweetly when the Gryphon Trio's Patipatanakoon plays it in a demonstration of the innovative instruments.

Light and lively. Balsa-wood violins crank out the sound.

Curtin's violin looks positively conventional next to the creations of Doug Martin, a boat builder and amateur violinmaker from Eliot, Maine. Strange, asymmetrical assemblages of unfinished balsa wood, Martin's violins look much like primitive folk art. Yet they pump out sound, and in spite of some rough qualities, they've captured the imagination of professional makers. Discarding the notion that a violin should look like a violin, the soft-spoken Martin has clearly let his imagination run. "These aren't models for a final product," he says. "They're purely experimental, for gaining experience as fast as you can." Some professional violinmakers feel that the homemade instruments can teach them something, too.

Visualize the vibe. A violin's body oscillates in a variety of patterns, or modes. Here, the top moves in a butterfly-shaped pattern, with opposite quadrants moving in opposite directions.

Top down or bottom up?

To be sure, some makers bristle at the idea of innovation. William Fulton of Idyllwild, California, questions whether a carbon-fiber or balsa-wood instrument counts as a violin. "It represents a new instrument that looks like a violin and it plays like a violin," he says, "but it ain't a violin."

Others worry that the use of carbon-fiber composites will inevitably lead to mass production of instruments. But cheap wooden violins are already mass-produced in China and elsewhere, and factories are cranking out ever better instruments, says Gregg Alf, a violinmaker in Ann Arbor. "Innovation is our defense against mass production," Alf says. "It allows us to offer something more than a factory that's 5 years behind."

Ultimately, musicians will decide whether innovative violins succeed. But no one knows what it will take to persuade a soloist to play Carnegie Hall with an ultralight violin. Some say it's simply a matter of getting superior instruments into the hands of leading violinists. "I suspect there's an underground lake of anger at having to pay so much money and having so many problems with [old] instruments," Curtin says, "so that if there's something better, [musicians] will change fairly quickly."

Others predict that change will begin at the bottom, with instruments for students. Student instruments are often so poorly made that it's nearly impossible to wring a decent sound from them, says D'Addario's Tao. Lightweight carbon-fiber instruments would be easier to play, he says, and if students grow up with innovative instruments, they may be more receptive to them as adults.

At least a few players are already willing to consider novel instruments. "I don't think anyone is willing to discount anything anymore," says violinist Patipatanakoon. In the end, what matters is how an instrument plays, she says, and she praises one of Martin's rough-and-ready balsa violins. "It's so comfortable," she says. "You can just sink into it."

Still, when asked which of the several instruments suits her the best, Patipatanakoon chooses one made of traditional materials by Andrew Ryan of Providence, Rhode Island—the most conventional one of the lot. A revolution in violinmaking may have begun, but it seems there's a tune in the old fiddle yet.

–ADRIAN CHO

CREDITS (TOP TO BOTTOM): A. CHO/*SCIENCE*; ADAPTED FROM MARTIN SCHLESKE

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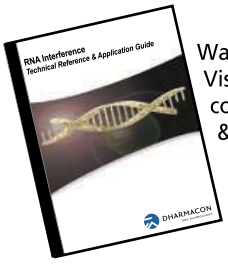

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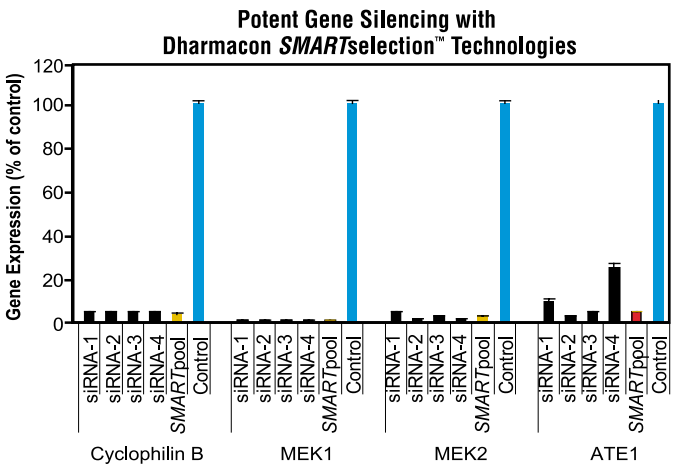

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The Congressman With His Hand On Science's Purse Strings

A veteran House member and chair of a key spending panel has become a cheerleader for U.S. research and science education

Once Frank Wolf takes up a cause, says his congressional colleague and good friend Sherwood Boehlert, it quickly becomes a passion for him. "He doesn't flirt with an issue. He marries it," quips Representative Boehlert (R–NY), chair of the House Science Committee, about the Virginia Republican who this year became head of the House spending panel that oversees more federal research agencies than does any other appropriations subcommittee.

In this case, the cause is the need to strengthen the U.S. scientific enterprise, from the classroom to the laboratory to the tax code. Next week will mark Wolf's arrival as a heavyweight on the national science policy scene. On 6 December, the federal government will sponsor a National Summit on Competitiveness, a 1-day event in Washington, D.C., at which dozens of business, government, and university leaders will be asked to throw their weight behind recommendations made in a slew of recent reports on the subject (usinnovation.org). The proposals include big increases in research spending, a special pot for risky ideas, training thousands of new science and math teachers, and increasing the number of students going into scientific careers. Wolf was a prime mover for the conference, giving the Commerce Department money to organize it.

Wolf's influence over science stems from his ascension in February to the chair of an expanded spending panel that, on the science front, gained authority over the budgets of NASA and the National Science Foundation (NSF); the panel already oversaw the budgets of the Commerce Department's National Institute of Standards and Technology and the National Oceanic and Atmospheric Administration. That reshuffling put Wolf in charge of a sizable chunk of government funding for civilian nonbiomedical research. He quickly used his new status to pen a 3 May letter to President George W. Bush calling for a tripling of federal spending on basic research over the next decade.

A lawyer and former political appointee in the Nixon and Ford administrations, Wolf, 66, entered Congress in 1981 on his third attempt, riding the long presidential coattails of Ronald Reagan. Reelected by comfortable margins by a combination of Washington suburbanites and rural Virginians, Wolf has been a loyal Republican and solid legislator who can be counted on to speak up for the many federal workers in his district. He's best known nationally for his outspoken advocacy of global human rights and has made several trips to Africa, the Middle East, and Tibet, where he has loudly denounced China's treatment of its underclass.

From the chair. Representative Frank Wolf has taken a keen interest in science spending.

"He's probably the most decent human being in the U.S. Congress," says former Representative Bill Goodling (R–PA), now a higher education lobbyist. "He's also tenacious. And I hope that the White House will recognize that he can be a major ally in trying to keep us cock of the walk in science."

Wolf's influence over science is limited, however, by how much money his committee is given to spend by congressional leaders and by the competition for those funds: Wolf's committee also handles the budgets of the Justice and State departments, for example. Still, during a tough year for discretionary programs, Wolf managed to persuade fellow legislators to give NSF not only more than the president requested but also more than the House had originally approved (*Science*, 11 November, p. 956).

Wolf admits that he was mindful of his new constituency as he negotiated the fiscal year 2006 budget. "After all the speeches I've given about increasing funding for science and education, had we not increased the NSF budget … It would have sent the wrong signal."

On 17 November, Wolf spoke with *Science*'s Jeffrey Mervis and Eli Kintisch from his Capitol Hill office in the Cannon building. What follows is an edited transcript of his comments.

When you meet with scientific groups, what do they want?

They are concerned about more resources and more emphasis on math and science education. I think that most people think we've either stalled or are falling behind.

You can feel that there's something that's not right. It doesn't take a rocket scientist to understand that. … Just look at the number of engineers being trained. China is turning out 500,000 a year, and India 350,000. And we're graduating 70,000.

Given the high unemployment rate among U.S. engineers, if we train more engineers, where would they work?

Well, I think they'd work at American companies. That would be one of the tradeoffs. If we train more people, you have to make sure that our companies hire them and not give the jobs to scientists overseas. And I think there will be a great need for them.

Do high-tech CEOs say that they will hire more scientists?

That's why we're having this summit. … I think that you have to educate the American people about how serious this is. And then you have to educate the leadership.

CREDIT: CHRIS MADDALONI

What would you like the Bush Administration to do?
Ideally, I would like to see the Administration paint of vision of where we need to be going. I guess that's what is lacking: the failure of a vision. … It should be similar to what President Kennedy did for sending a man to the moon, and what President Eisenhower did after Sputnik. Everybody knows what is missing. But you need to explain what this means to the country. Or do you want all the jobs to leave this country, and have China become the dominant world power? The Chinese government is spying against us, and its government is throwing Catholic priests and Protestant clergy in jail and is plundering Tibet. Is that who you want to lead the world in innovation? I think most people would say no. …

This country needs to do something to make sure that the jobs don't go abroad, that we do something to improve math and science education. We have to pay teachers more and make sure our youngsters have real math and science teachers. You can't stop the opposition. You can't stop a youngster in Romania from wanting to be involved in the American dream. So we have to make sure that we have good students, well-trained teachers, great universities, and the proper tax policy to foster research.

Speaking of teachers, do you think that the debate about intelligent design …
I'm not going to go there. I don't think that it's even part of this process. We're talking about funding. …

But many scientists are worried that it might affect public attitudes toward science.
No, I don't think so. … What's going to make a difference is improving science and math education. I think that ID is a news story, and it's something that journalists like to write about. But it's not the real issue.

Why doesn't the Administration share your vision [on what the U.S. needs to do to remain competitive]? Do they disagree with you, or do other things have a higher priority?
I think they are bogged down with other things. The war against terrorism is very important. Recovering from Katrina and Rita, too. And I don't know that up until earlier in the year, that there were quite the facts [available] to describe the problem. … But my sense is that now, I'm hopeful that this will become a priority in the [next] budget that's submitted. You can't just do things with words. You need deeds, too. … Most of what has to be done should be relatively noncontroversial. The costs are not large.

–JEFFREY MERVIS AND ELI KINTISCH

On show. The new fossil of *Archaeopteryx* will join this *Camarasaurus* and other Jurassic dinosaurs in the Wyoming Dinosaur Center.

Best *Archaeopteryx* Fossil So Far Ruffles a Few Feathers

By acquiring a dream fossil, a privately owned museum hopes to boost its scientific reputation—but some paleontologists remain skeptical

When the first *Archaeopteryx* fossil emerged from Bavaria's Solnhofen limestone in 1861, the slab of rock electrified the scientific world. Sporting birdlike feathers but the teeth and tail of a dinosaur, the magpie-sized creature shed light on the origin of birds and bolstered defenders of Darwin's *Origin of Species*, published just 2 years before. Over the next century, six more skeletons turned up in the same rocks and won pride of place in some of the most prestigious natural history museums of Europe.

Now there is one more in the flock, and it's causing a flap. On page 1483, paleo-ornithologist Gerald Mayr of the Senckenberg Natural History Museum in Frankfurt, Germany, and colleagues describe the best preserved *Archaeopteryx* yet. "By all measures, it is a treasure," says Peter Dodson of the University of Pennsylvania. Unlike its predecessors, however, this one is heading not for a major urban museum but to a small, privately owned museum in Thermopolis, Wyoming (population 2953). And some paleontologists say it deserves better.

The new specimen is undeniably world-class. The skull further links *Archaeopteryx* to its close dinosaur relatives. The foot is also better preserved than that of previous specimens, and it shows a hyperextensible second toe—like the killer claw of *Velociraptor*—and appears to have been best suited for life on the ground rather than in the trees as some had supposed. Although there's nothing radically new about the specimen, "it's the dream of every paleo-ornithologist to describe an *Archaeopteryx*," Mayr says. "We would have loved to have the specimen in Frankfurt."

Instead, the rara avis will alight in the Wyoming Dinosaur Center, founded in 1995 by Burkhard Pohl, an independently wealthy former veterinarian with a lifelong interest in fossils. Some paleontologists object. "There's no guarantee that it will be preserved and curated in perpetuity," says Mark Goodwin of the University of California, Berkeley's, Museum of Paleontology. Goodwin and others are also leery of Pohl's connections to the world of commercial fossil dealing, an activity that they argue undermines scientific research (*Science*, 14 April 2000, p. 238). Both the Society of Vertebrate Paleontology and its journal have policies strongly discouraging the study of privately held fossils.

But Mayr and other scientists say that the specimens at the Wyoming Dinosaur Center are too good to ignore and that Pohl has made efforts to beef up his institution's scientific expertise. "They really are striving to establish a level of credibility," says Brent Breithaupt, who heads the University of Wyoming Geological Museum in Laramie.

CREDIT: COURTESY OF WYOMING DINOSAUR CENTER

Pohl, 49, grew up with an interest in natural history. After earning a Ph.D. in veterinary medicine at the University of Berne, Switzerland, Pohl worked in a virology lab, but fossils were his real interest. With a sizable inheritance from his family's cosmetics and hair-care company, Pohl began to expand his collection.

In the early 1990s, while traveling through the Big Horn Basin of Wyoming, Pohl heard about a ranch near Thermopolis with dinosaur-rich outcrops. Pohl and his then–business partner, a German fossil preparator and dealer, negotiated a lease to dig for fossils. When the 3035-hectare property came on the market in 1993, Pohl bought it for $800,000.

Their company, Big Horn Prospecting Inc., was set up as a for-profit business to excavate dinosaurs. The partners soon split up, and Pohl decided to set up a museum that would show fossils being dug up and prepared, as well as exhibits of casts and real skeletons. In July 1995, he opened the Dinosaur Center, a 1500-square-meter steel building. "It's not the prettiest structure," he admits. Pohl also set up the nonprofit Big Horn Basin Foundation to help care for the fossils, run the exhibits, and take tourists, for $125 a head, out to dig at some of the sites.

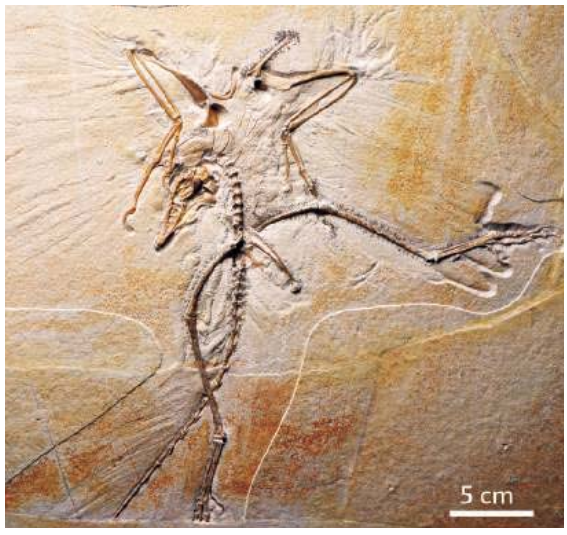
As museums go, the center is a shoestring operation. About a dozen people, including three or four preparators, work there year-round. Pohl holds the only Ph.D. In May, he hired Scott Hartman as science director. Hartman has a bachelor's degree in zoology, training in scientific illustration, and several years of museum experience. This year, Hartman and his colleagues presented findings at the Society of Vertebrate Paleontology's annual meeting, including the oldest known specimen of a troodontid (see *Science*NOW, sciencenow.sciencemag.org/cgi/content/full/2005/1021/1).

Pohl also makes specimens in his collections available to outside scientists. In July 2004, Eric Buffetaut of CNRS in Paris and David Martill of the University of Portsmouth, U.K., published a paper in *Nature* that showed that a fish-eating dinosaur called *Spinosaurus* ate pterosaurs too. The Brazilian specimen—a tooth embedded in a pterosaur vertebra—is housed at the center. "I do not describe fossils in private collections, if I can help it," says Buffetaut, but he says he has no misgivings about fossils in the Wyoming Dinosaur Center. "I know who's in charge, and I trust him to behave in a scientifically acceptable way."

But some paleontologists remain uncomfortable about working with Pohl. They want to be absolutely certain that fossils, particularly foreign ones, were legally excavated. China and Mongolia, for example, have spectacular vertebrate fossils—and laws against exporting them. Pohl says that the center has never bought anything from these countries, but he himself has purchased specimens with an unclear history, because they looked scientifically significant. "If I know something is stolen, I won't touch it," he says. But he acknowledges, "it's really gray lots of times."

Scientists also worry about whether the center can guarantee them future access to scientific specimens, as mainstream museums can. Privately owned specimens can be sold. Although some of the center's fossils, including the *Spinosaurus* tooth, are officially owned by the Big Horn Basin Foundation, others belong to Big Horn Prospecting or to Pohl himself. Hartmann says he doesn't know exactly how many of the collection's 10,000 bones fall in each category. The center is overhauling its collections database and management system, he says, to make curatorial information more accessible and track issues of ownership.

The origins of the *Archaeopteryx*, however, remain hazy. Pohl says he "found a donor" to buy it from a private collector after the Senckenberg failed to raise enough money. (Mayr declines to reveal the asking price, but the Paläontologische Museum München paid DM 2 million—about $1.3 million—for a less spectacular specimen in 1999.) The *Archaeopteryx* appears to be legal, because Bavaria allows the export of fossils.



Prize bird. This new specimen of *Archaeopteryx* is one of the best preserved and the first to go to a museum outside Europe.

Pohl won't say who legally owns it, but he says that it's "guaranteed that it will stay in a public collection."

That's not good enough for some paleontologists, who recall ruefully how another privately owned *Archaeopteryx* went missing after its owner died in 1992. They worry most about so-called type specimens—the original reference fossils for new species. Pohl himself owns the type specimen for a new species of crane, *Parvigrus pohli*, which Mayr described in the 29 July online issue of *Naturwissenschaften*. "Having a holotype in a private collection is quite questionable," says Luis Chiappe of the Natural History Museum of Los Angeles County in California. Kevin Padian of the University of California, Berkeley, goes further: "No respectable journal would publish a specimen that was not in a permanent public repository."

The critics say their fears would be eased if the center were officially accredited by the state or federal government. Pohl and Hartmann say that the center already abides by many guidelines. "The policy that we have is that the first and best of every specimen should stay in the collection," Pohl says. After he dies, Pohl says, he wants the collection to stay together, but he hasn't worked out the details yet. If his family is any indication, there's reason to believe. Last year, Pohl's mother, a Ph.D. biologist and lifelong mineral collector, donated her collection of more than 80,000 minerals to the Technische Universität Bergakademie in Freiberg, Germany—making its collection the largest in the world.

—ERIK STOKSTAD

Digging in. Burkhard Pohl, owner of the Wyoming Dinosaur Center, helps out with an excavation on his ranch.

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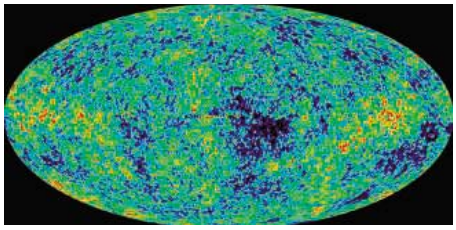
Random Samples

Edited by Constance Holden



Cosmic bulletin board?

Founder's Message

Combing through cosmic radiation could reveal a message from the universe's creator, if it has one, say two physicists.

According to theory, anyone could make a universe by squashing a lump of matter violently enough to replicate the big bang. And by tweaking something called the inflaton field, the creator—be it a physicist-hacker or a deity—could put a binary message in the cosmic microwave background (CMB) radiation. Or so argue Stephen Hsu of the University of Oregon, Eugene, and Anthony Zee of the University of California, Santa Barbara, in a paper at arXiv.org. The message might sit, like cosmic Braille, in the bumps and ripples of the CMB, they say. They calculate that it could hold up to 100,000 bits of information—enough to encode, say, clues to the long-sought grand unified theory that joins all the physical forces. Some people "think we are nuts," says Hsu. "I think it's a legitimate scientific question." Telescopes now in the works could detect such a message within 20 years, he says.

There's a hitch, though, says cosmologist Douglas Scott of the University of British Columbia in Vancouver, Canada: Observers in other times or parts of the universe would see different patterns, so the creator would have to specify a time and place for deciphering the bumps.

Neolithic Cattle Go Wild

The 9500-year-old farming settlement of Çatalhöyük, in Turkey, which has spectacular wall paintings and sculptures of bulls, has long been considered the site of the first known domesticated cattle. But a new analysis of cattle bones at the site suggests it's not.

The claim was based on a 1969 *Science* paper by the late zooarchaeologist Dexter Perkins, who argued that the bones were not as large as those of wild cattle. But a new team of faunal experts led by Nerissa Russell of Cornell University and Louise Martin of the Institute of Archaeology at University College London has examined 4321 bone pieces. Relying not just on size but also sex and age patterns, which differ between hunted and herded animals, they conclude in the December issue of *Current Anthropology* that the cattle were wild during at least the first three-quarters of the 1200-year life of the settlement.

Zooarchaeologist Simon Davis of the Portuguese Institute of Archaeology in Lisbon says the conclusion is "a little strange" in light of recent evidence from Cyprus suggesting that cattle were herded there more than 10,000 years ago, as well as slightly later signs of domestication at other Near Eastern sites. But zooarchaeologist Melinda Zeder of the Smithsonian Institution in Washington, D.C., says the new findings are "solid" and "more compatible" with the evident symbolic status of the animals, whose horns and skulls adorn many of Çatalhöyük's mud-brick houses. "Elsie the cow hardly makes an impressive cult figure," she says.

Early cattle installation in Çatalhöyük.

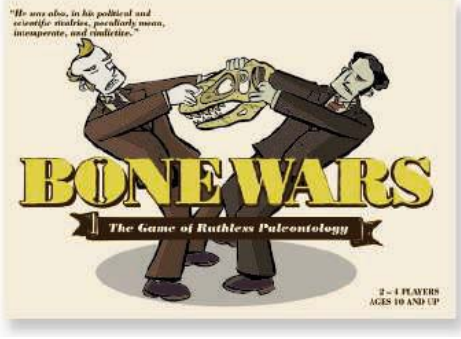
Attachment Chemistry

Evidence has been growing that emotional deprivation early in life can permanently change people's brains. Now a group at the University of Wisconsin, Madison, reports that children who suffered early neglect in orphanages have deficiencies in hormones related to attachment.

The hormones—oxytocin, whose levels increase with warm physical contact with a familiar person, and vasopressin, which plays a role in recognizing familiar people—are hard to measure. But psychologist Seth Pollak and colleagues say they have devised a way to get measurements from urine, making it possible to test small children.

Eighteen tots who had spent an average of 16 months in orphanages before adoption were compared with 21 children raised by their biological parents. Over a 2-week period, each child had two cuddly 30-minute play sessions, one with his or her mother and one with an unfamiliar woman. The researchers found that in the family-reared children, oxytocin levels increased after contact with the mother, but not with the stranger. But oxytocin levels never went up in the orphans, who also had lower baseline vasopressin levels, the researchers reported last week in the 22 November issue of the *Proceedings of the National Academy of Sciences*.

Thomas Insel, director of the National Institute of Mental Health in Bethesda, Maryland, says the report is consistent with not only animal evidence but also evidence that children with autism—who avoid social interactions—also lack oxytocin responses. If the urine-sampling method proves valid, he says, "it could jump-start a new approach to clinical studies."



Merry Christmas, Kansas

Now that the Kansas Board of Education has redefined "science" to include the supernatural, this paleontology game's makers are fighting back, offering a 20% discount to anyone in Kansas. Bone Wars (www.zygotegames.com) teaches players how to form and test hypotheses as they pretend to be "ruthless paleontologists" from the late 1800s U.S. "Dinosaur Rush."

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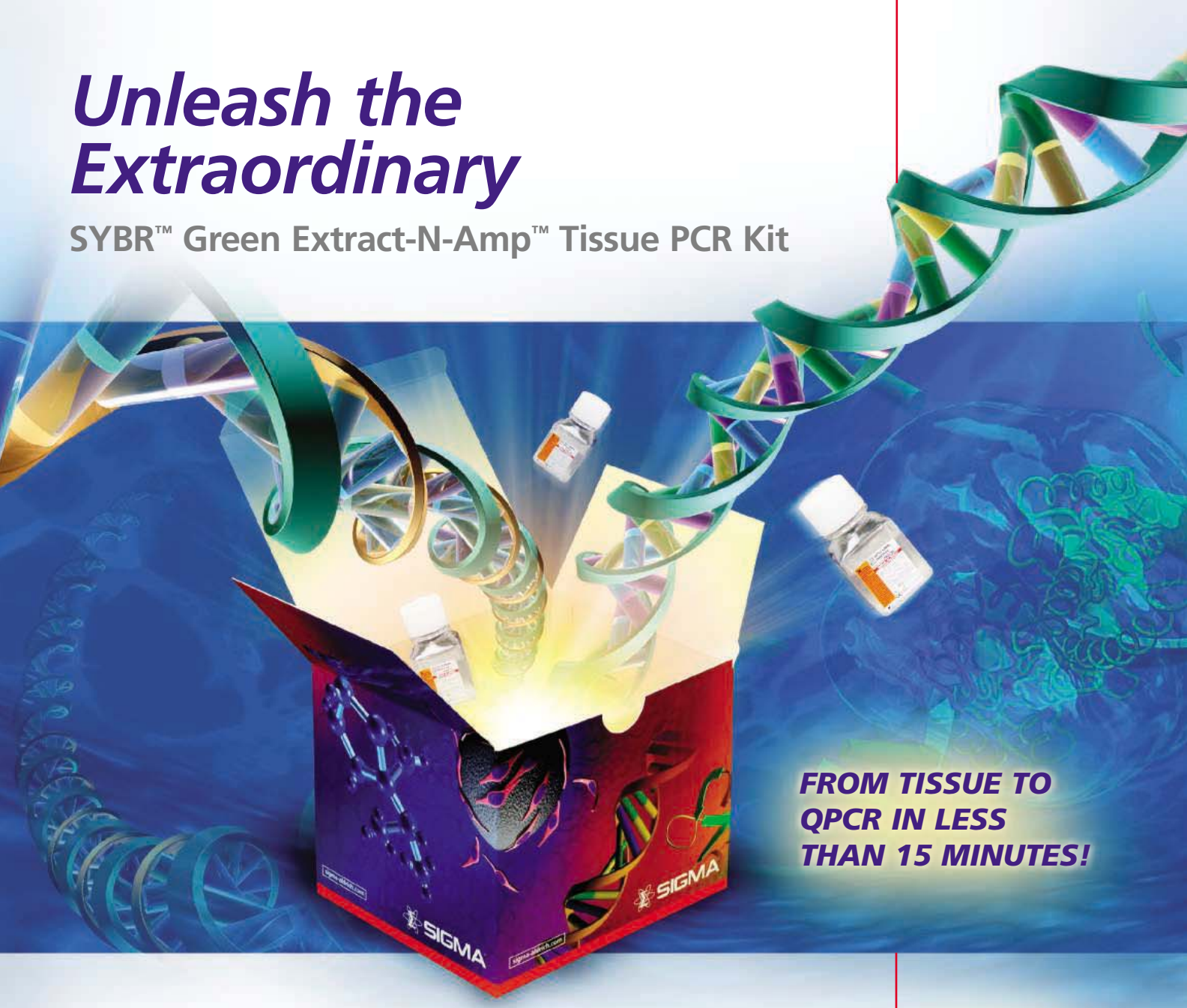

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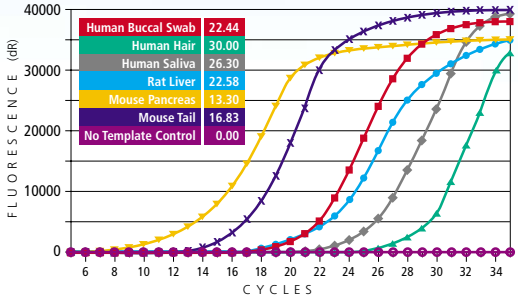

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Edited by Yudhijit Bhattacharjee

FACE OFFS

Wielding the knife. If you can't cure it, cut it. That appears to be how the National Cancer Institute is dealing with a pain-inducing newsletter that has criticized NCI chief Andrew von Eschenbach.

Last week, NCI cancelled its subscription to *The Cancer Letter*, an independent journal in Washington, D.C., that has published some scathing articles on NCI leaders, including recent allegations that von Eschenbach has a conflict of interest because he is trying to direct NCI and the Food and Drug Administration at the same time (*Science*, 7 October, p. 29). *The Cancer Letter* publisher Kirsten Goldberg and editor Paul Goldberg—a husband-wife team—say NCI's cancellation of its site license means a loss of 600 dedicated readers at the institute and a $48,000 revenue loss that will force the journal to cut costs.

The cancellation of *The Cancer Letter* and two other "nonscientific" newsletters is "due to budgetary constraints," says NCI spokesperson Nicole Saiontz, and not because of any unhappiness with its coverage. NCI employees will still be permitted to use program funds for subscriptions, she says.

But as one staffer notes, NCI could end up spending more than it saves if a lot of scientists take that approach.

AWARDS

Nation's pride. Economist Kenneth Arrow, plant pathologist Norman Borlaug, and biochemist Phillip Sharp—all Nobel laureates—are among the eight winners of the 2004 National Medal of Science, announced by the White House last month. The other winners are transplant surgeon Thomas Starzl of the University of Pittsburgh School of Medicine, chemist Stephen Lippard of the Massachusetts Institute of Technology, biochemical engineer Edwin Lightfoot of the University of Wisconsin, Madison, mathematician Dennis Sullivan of Stony Brook University in New York, and geochemist Robert Clayton of the University of Chicago.

The White House also named two individuals and five companies as winners of the 2004 National Medal of Technology: Ralph Baer for his pioneering role in the development of interactive video games; Roger Easton for contributions to spacecraft tracking, navigation, and timing technology; Gen-Probe Inc. for developing new blood testing technologies; the microelectronics division of IBM for advances in semiconductors; Industrial Light and Magic for innovations in visual effects technology; Motorola for leadership in the communications industry; and Paccar Inc. for developing and commercializing aerodynamic, lightweight trucks.

CHECKING IN

Political science. Working on biodiversity issues at the U.S. Agency for International Development and the National Institutes of Health, botanical systematist Francesca Grifo learned that policymaking is often guided by factors other than peer-reviewed science. She hopes to reduce the chances of that happening as director of a new permanent program on scientific integrity at the Union of Concerned Scientists (UCS).

Grifo most recently worked as a policy instructor at Columbia University and curator of the American Museum of Natural History. She came to UCS this fall to make science a stronger force in the political arena. "Just because you're right and just because you have your data doesn't mean science takes the day," she says. Among the challenges she wants to tackle are inadequate protections for whistleblowers, questionable appointments to federal scientific advisory boards, and the role of science in decision-making. To those who label UCS as partisan and liberal, she says "we're focusing on this Administration because that's what's happening now."

POLITICS

One degree too hot? Just a year after taking office, Ireland's first national science adviser, Barry McSweeney, has been transferred to a new job in light of a controversy over alleged flaws in his résumé. McSweeney had described himself as a "biochemist" with a Ph.D. from Pacific Western University (PWU) in Los Angeles, California. Questions about his credentials arose after an investigation last year by the U.S. Government Accountability Office (GAO) named PWU as one of several "diploma mills" that give degrees based on life experience rather than coursework. PWU, for example, offered to sell a Ph.D. to a GAO investigator for $2595 (www.gao.gov/new.items/d04771t.pdf).

McSweeney, 55, has two degrees that haven't been challenged: a bachelor's from the University of Cork and a master's from Trinity College, Dublin. Before becoming science adviser, he directed a joint research center for the European Union, overseeing a staff of 2500 in four countries. Last week, he moved to a research manager's job in the Irish Department of Communications, Marine, and Natural Resources.

"Nobody questioned [McSweeney's] ability or enthusiasm," says Conor O'Carroll of the Irish Universities Association in Dublin, but O'Carroll says some people felt his résumé was not a good advertisement for Irish science. McSweeney's former office issued a statement saying he was "pleased" with his new job but that he would not comment further.

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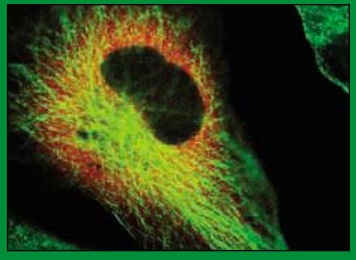

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Issues in Bringing New Drugs to the Market

THE EDITORIAL "SENDING PHARMA BETTER signals" by J. Avorn (29 July, p. 669) provides a good outline of what should be done to enhance the development and subsequent use of pharmaceutical medications. Oversight by regulatory agencies, purchasing by government and third-party payors, physician prescribing, advertising to the public, and patient usage all demand attention to enhance the future development of pharmaceutical products. One area that should be added is postmarketing surveillance to ensure the efficaciousness and lack of harmful effects of the additions to the therapeutic marketplace.

RUDI ANSBACHER
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J. AVORN'S EDITORIAL "SENDING PHARMA better signals" (29 July, p. 669) points out that the pharmaceutical industry has been one of the most profitable in American history. This is because they are successful and because they have been given more government incentives than most industries, including extensive patent protection. They have also been able to link directly to extraordinary scientific discoveries within academic medicine. The real problems are that they are spending too much on advertising, are developing "me too" drugs to bypass patent issues, are lax in postmarketing surveillance for safety issues, and are not investing enough of their profits into research for new drugs. It's time for the pharmaceutical industry to get serious about research and take more of the billions spent on advertising and put it into research for new drugs.

ARTHUR J. AMMANN
Clinical Professor of Pediatrics, University of California San Francisco Medical Center, 104 Dominican Drive, San Rafael, CA 94901, USA.

IN HIS EDITORIAL "SENDING PHARMA BETTER signals" (29 July, p. 669), J. Avorn provides several recommendations for "rescuing" the pharmaceutical industry. He begins by criticizing a drug industry advertisement. Avorn seems not to understand a simple economic fact of life: If there is to be a next generation of drugs, it can only be funded from the sales of today's medicines. Avorn calls the number of new drugs "disappointing" and I can only agree. But I disagree with his diagnosis.

There are many reasons for the paucity of new drugs, and in the same issue of *Science* in which the Editorial appears, we can find some of the more important ones (see Special Section on Drug Discovery, pp. 721–735). But the most obvious reason is that we have dared to reach high. Never before has humanity sought to treat diseases as complicated and refractory as those we have chosen to target today. They are the most challenging; they present us with the greatest risk of failure, and sadly, failure is often what we see.

Avorn suggests changing the patent law to protect "the basic science that undergirds so much of what the industry does." This proposal is puzzling because the patent system already protects "basic science," just as it protects any inventor whose work meets the requirements of the law. Avorn fails to mention the Bayh-Dole Act, which for the past 25 years has brought about enormous growth in the number and value of patents issued for taxpayer-funded work in "basic science." These inventions are not drugs, but are instead insights, approaches, and tools, and the pharmaceutical industry is a regular customer for them.

> **"If there is to be a next generation of drugs, it can only be funded from the sales of today's medicines."**
>
> –TRACEY

Avorn proposes that the U.S. Food and Drug Administration's (FDA) advisory committees be made to comment on the importance of a new drug's therapeutic contribution. This proposal either does no more than restate what the FDA review process already does—balance safety and efficacy in the context of available alternative treatments—or else it demands a vast expansion of comparative drug studies to arrive at some "deeper" understanding of how much value a new drug would bring to patients. If, as Avorn remarks, new drugs are already disappointingly few, his "solution" would only add to the cost, delay, and attrition. Moreover, whether a new drug offers "enough" added therapeutic benefit begs the question "therapeutic benefit for whom?" At the level of individual patients, even a "modest" therapeutic advance in a drug class can mean the difference between a drug that works and one that doesn't. Patients and their physicians should decide which drug is most appropriate for the patient, not the government.

Finally, Avorn is incorrect when he states that Medicare cannot consider a drug's therapeutic value or cost-effectiveness during the implementation of the new drug benefit; the Medicare Modernization Act does not prevent this evaluation, and it would be surprising if the private insurance companies administering these benefits under the Medicare program did not consider these factors when determining their formularies.

The signals being conveyed loud and clear to the pharmaceutical industry, and drug discovery scientists like myself, are that the industry is not valued, brings minimal benefit to society, and is in fact solely focused on profits over the welfare of patients. My experience in the industry tells me that nothing could be farther from the truth. Unfortunately, the industry assumes that the public understands the difficulty and expense of the work we do, while the public, accustomed to the benefits of past advances in health care, has come to assume these miracles are quick, cheap, and easy—but they are not. All of us in the scientific community need to ensure a rational debate about the role of the pharmaceutical industry in our current health care crisis, for if we fail, the future of drug discovery and development in this country is bleak.

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Response

ANSBACHER IS CORRECT THAT BETTER POST-marketing surveillance is a key ingredient in bringing more science-based evaluation to clinical drug choices, and I agree with Ammann on the need to redirect more pharmaceutical dollars into important drug research rather than advertising. Tracey's Letter contains several statements with which I cannot agree. He reiterates the industry mantra that all future progress in developing new drugs relies on lucrative present-day pharmaceutical sales, despite the fact that the record profits of the last decade have not been transformed into the expected return in new drug products. There is also compelling evidence that many important drug discovery breakthroughs rely instead on the findings of publicly funded research conducted in academic centers (*1*). Such basic science is not protected as well as Tracey suggests; in recent years, the courts have often denied patents to university scientists who made

pivotal discoveries on which new drugs were based, leaving all intellectual property rights in the hands of the companies that exploited those findings to create commercialized products (*2*). He is incorrect in stating that the current FDA approval process provides adequate perspective on the relative worth of a new drug; usually, it merely determines that a new product works better than a placebo at achieving a short-term surrogate outcome—hardly all the information a clinician or payor needs (*3*). The "vast expansion of comparative drug studies" that Tracey fears is precisely what the nation requires to help us make drug prescribing and purchasing decisions more wisely and to bring some badly needed discipline to the drug development and marketing process. I agree that there is nothing wrong with "modest" or patient-specific therapeutic advantages, as long as they are real rather than illusory. We just need to define them better and decide how much patients or society are willing to pay for them. Finally, Tracey comments on the government's abdication of responsibility for assessing the value of drugs to be covered under the new Medicare prescription benefit program. True, the new law does not prevent such evaluation. It also does not prevent universal health coverage, but that doesn't mean that anyone will step forward to make that happen either. Relying on private insurance companies to provide this public good will likely be as disappointing as our earlier reliance on that industry to contain drug costs and ensure affordable medical care.

> **"[T]he record profits of the last decade have not been transformed into the expected return in new drug products."**
>
> –AVORN

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Invariant Ratios Vs. Dimensionless Ratios

IN THEIR REPORT "THE ILLUSION OF INVARIANT quantities in life histories" (19 Aug., p. 1236), S. Nee *et al.* show that purely statistical methods may lead us to conclude that certain life-history ratios are invariant when, in

fact, they are not invariant at all, but the statistical procedure—in which one regresses $X + c$ on X—causes one to think so.

Some confusion may have also occurred in this field because of the difference between a dimensionless ratio and an invariant one. A simple case, originally attributable to Beverton and Holt (*1*), can illustrate the point. If an organism grows according to the von Bertalanffy form $L(t) = L_\infty(1 - e^{-kt})$, where t is age, L_∞ is asymptotic size, k is the growth rate, survival to age t is e^{-Mt} (where M is the rate of mortality), and fitness with maturity at age t is $e^{-Mt}L(t)^b$ (where b is the allometric parameter connecting size and fecundity), then it is an exercise in introductory calculus to show that the optimal age of maturity is $t^* = (1/k) \log [(M + bk)/M]$ and that the relative size at maturity is $L(t^*)/L_\infty = b/[b + (M/k)]$. The ratio M/k is dimensionless but need not be invariant. However, for any two species in which this ratio is the same, the relative size at maturity will be the same.

I suggest that it might be more productive for us to follow the example of fluid mechanics and replace the notion of invariants by explicit dimensionless numbers. Define, for example, the "Beverton number" $\nu_B = M/k$ so that $L(t^*)/L_\infty = b/(b + \nu_B)$. Then we conclude that for species in which $\nu_B \to \infty$, relative size at maturity will be very small, whereas for those species in which $\nu_B \to 0$, relative size at maturity will be close to asymptotic size. Life-history invariants may be elusive, but dimensionless numbers and their life-history consequences are not.

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Center for Stock Assessment Research and Department of Applied Mathematics and Statistics, University of California, Santa Cruz, CA 95064, USA.

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Worldwide Decline of Sturgeons

IN THE ARTICLES "U.S. TO BAR CAVIAR" (Science Scope, 16 Sept., p. 1799) and "Ban on beluga caviar points to sturgeon's worldwide decline" (News of the Week, 7 Oct., p. 37), C. Pala reports that the United States has banned the importation of caviar from the beluga sturgeon to protect this very old species. The sturgeon order Acipenseriformes already faces local extinction for 19 species. A worldwide boycott on beluga caviar and control of domestic markets are suggested, because overfishing is held to be responsible for the decline. The article by R. Stone "The sturgeon's last stand" (News Focus, 16 Sept., p. 1806) on breeding facilities for Caspian sturgeons in Rasht, Iran, discusses efforts to boost sturgeon stocks. These measures are, however, insufficient. In addition to poaching, pollution destroying spawning habitats and human interventions, which prevent migration to spawning grounds, have been blamed (*1*). Another factor is uncontrolled restocking, drastically reducing genetic diversity, as observed in the Volga River, habitat of the Russian sturgeon *Acipenser gueldenstaedtii*. Here, 11 of 34 sturgeons morphologically classified as *A. gueldenstaedtii* had haplotypes of *A. baerii*, originally confined to Siberia (*2*). The interdependence of environment and genetics is demonstrated by a remarkable species shift, occurring in the Baltic between 800 and 1200 A.D., when the North American sturgeon, *A. oxyrinchus*, replaced the native sturgeon, *A. sturio*, because of a drastic drop in water temperature during the little ice age, favoring *A. oxyrinchus*, which spawns below 17.8°C, over *A. sturio*, which spawns above 20°C (*3*).

Another detrimental factor is aquatic hypoxia, which, because of pollution, is on the rise worldwide (*4*). Hypoxia disrupts reproduction in teleost fish (*5*). Having a much higher oxygen consumption per kg per hour than teleosts (*6*), sturgeons may even be more susceptible to reduced oxygen levels. Coping with oxygen depletion by reducing total energy expenditure after mild reduction of PO_2 (*7*), and by bradycardia, hyperventilation, and a shift to anaerobic metabolism during severe hypoxia (*8*), sturgeons have acquired similar adaptive mechanisms as more advanced fish. However, even brief periods of moderate hypoxia (PO_2 = 30 mm Hg, 30 min) have a detrimental effect upon the sturgeon's central nervous system, leading to significant cell death (apoptosis), mainly in the spinal cord and the pituitary (*9*). To prevent the sturgeon's extinction, pollution and aquatic hypoxia must be reduced to prevent neuronal death and improve reproductive behavior.

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CORRECTIONS AND CLARIFICATIONS

Reports: "Metal-insulator transition in disordered two-dimensional electron systems" by A. Punnoose and A. M. Finkel'stein (14 Oct., p. 289). On page 289, third column, first line below equation 2, "where $\xi = -\ln(1/T\tau)$" should read "where $\xi = \ln(1/T\tau)$." On page 290, in the Fig. 1 legend, in the second line, "t=Θ" should read "t-Θ."

Research Articles: "Ultrafast dynamics of solute-solvent complexation observed at thermal equilibrium in real time" by J. Zheng *et al.* (26 Aug., p. 1338). The authors wish to acknowledge the contributions of Peter Hamm and co-workers [S. Woutersen, Y. Mu, G. Stock, P. Hamm, *Chem. Phys.* **266**, 137 (2001)], who were the first to use two-dimensional infrared (2D IR) spectroscopy to observe chemical exchange. Those authors employed a phase-insensitive pump-probe technique, as opposed to the fully coherent Fourier transform 2D IR vibrational echo method reported in *Science*.

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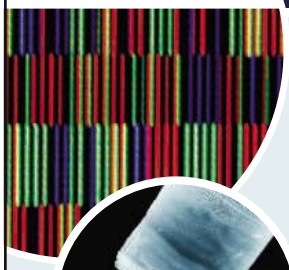

Enabling Science Through DNA Sequencing

The US Department of Energy Joint Genome Institute (JGI) has created the Community Sequencing Program (CSP) to provide the broad scientific community with access to high-throughput DNA sequencing. Based on scientific merit assessed by independent peer review, JGI will allocate up to 20 billion bases toward projects with high impact in the fields of microbial, environmental and plant genomics.

Through the CSP, the DOE aims to enable sequence based scientific research from a broad range of disciplines. The CSP consists of two programs: a small-genome program for shotgun sequencing of genomes smaller than 250 Mb and other sequencing projects with a total request of less than 1 Gb; and a large-genome program for shotgun sequencing of genomes larger than 250 Mb. Large-genome proposals should address DOE's broad mission including carbon sequestration, environmental remediation, and alternative energy production.

All steps in the proposal submission process will be conducted online via the JGI CSP website: http://www.jgi.doe.gov/CSP/index.html. Important deadlines are as follows:

- A Letter of Intent is required and must be submitted online between **December 1, 2005 and January 13, 2006.**
- Applicants will be notified by **January 20, 2006**, whether to submit a full Sequencing Proposal.
- Sequencing Proposals (with previously approved Letter of Intent) must be submitted online by **March 3, 2006.**

For additional details, please visit the JGI CSP website: http://www.jgi.doe.gov/CSP/index.html.
Please direct inquiries to CSP@jgi.doe.gov.

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Reports: "Rheological measurements of the thermoviscoelastic response of ultrathin polymer films" by P. A. O'Connell and G. B. McKenna (18 Mar., p. 1760). This paper reported the radii of curvature of the inflated nanobubbles as varying from approximately 600 to 3300 nm. The correct radii of curvature should be approximately 9000 nm (for the material in the glassy regime) to approximately 3300 nm (for material at the rubbery plateau). In addition, the caption for Fig. 2 reports the applied pressure as 0.7 MPa. The correct pressure is 0.07 MPa (as reported in the main text). Finally, on page 1763, it was noted that the prestrain required to account for the decreased compliance was 760% and that this was much less than the measured prestrain of 25%. This should instead read "much more than the measured prestrain of 25%." The authors thank H. Bodiguel and C. Fretigny for pointing out the error in our description of the radii of curvature and apologize for any confusion that these mistakes may have caused.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "The Great Sumatra-Andaman Earthquake of 26 December 2004"

S. Neetu, I. Suresh, R. Shankar, D. Shankar, S. S. C. Shenoi, S. R. Shetye, D. Sundar, B. Nagarajan

Lay *et al*. (Research Articles, 20 May 2005, p. 1127) estimated a 600-km length for the tsunami source region. Adding tide-gauge data from Paradip, the northernmost of the Indian east-coast stations and therefore the most critical constraint on the northern extent of the source, we estimate that its length was greater by ~30%.

Full text at www.sciencemag.org/cgi/content/full/310/5753/1431a

RESPONSE TO COMMENT ON "The Great Sumatra-Andaman Earthquake of 26 December 2004"

Thorne Lay, Hiroo Kanamori, Charles J. Ammon, Meredith Nettles, Steven N. Ward, Richard C. Aster, Susan L. Beck, Susan L. Bilek, Michael R. Brudzinski, Rhett Butler, Heather R. DeShon, Göran Ekström, Kenji Satake, Stuart Sipkin

We support the revised estimate of tsunami source length (~800 km) obtained by Neetu *et al*. Sea-level monitoring with a high sampling rate, good azimuthal coverage, and real-time access, along with detailed bathymetry data around the stations, would improve source region estimation from tsunami arrival times.

Full text at www.sciencemag.org/cgi/content/full/310/5753/1431b

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

NATURAL HISTORY

Still Worthy of Our Land?

Joel Greenberg

Return to Wild America
A Yearlong Search for the Continent's Natural Soul
by Scott Weidensaul
North Point Press, New York, 2005. 422 pp. $26, C$36.50. ISBN 0-86547-688-8.

In 1953, Roger Tory Peterson and James Fisher, two of the 20th century's premier naturalists, embarked on a 30,000-mile exploration of what Peterson, in the title of their account of the journey (*1*), called "wild America." They started at Cape St. Mary's, Newfoundland, largely to witness the huge colonies of gannets, murres, and kittiwakes (seabirds were Fisher's specialty). After flying to Boston, they drove the perimeter of the United States—down the Appalachians to the Everglades, around the Gulf Coast to Texas, through the desert Southwest, and north along the Pacific coast to Seattle. Along the way they added a foray into the Mexican tropics. They then took a plane to Alaska, the last destination of their 100-day-long grand tour. After returning separately to Seattle, Fisher flew east, while Peterson drove home via the interior, lamenting that Fisher didn't have the time to take in that part of the country as well.

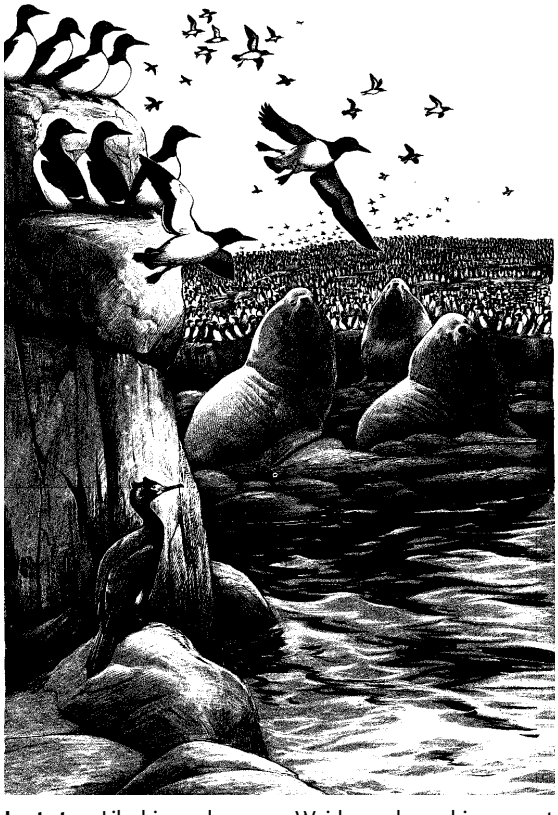
In *Return to Wild America*, Scott Weidensaul employs the clever technique of linking Peterson and Fisher's journey to what seems to be a disparate collection of essays on a wide range of hot conservation issues and exciting organisms. The book's ostensible purpose is to retrace the path taken by Peterson and Fisher 50 years earlier, but as the author admits, he used "their original itinerary as a broad framework…to visit places that best illustrate the changing landscape." In other words, he picked the locales to match the issues, rather than vice versa. *Wild America* includes a map with arrows showing the route, whereas the map in *Return to Wild America* shows merely the individual places that are discussed. Weidensaul—a writer whose previous books include accounts of avian migration (*2*) and the search for vanished species (*3*)—apparently did not take one long trip but instead made a number of shorter excursions (presumably spending intervening periods at home). By writing about a series of targeted trips to places that represent specific issues rather than actually driving around the country to see how things have changed since Peterson and Fisher's journey, the author failed to convey, at least to me, any sense of the discovery that actual exploration can provide. Weidensaul's "search for the continent's natural soul" (his book's subtitle) was mostly complete before he ever left home.

Although I am disappointed in the gulf between what the book claims to be and what it is, I found many of the discussions to be of great interest. A fine writer, Weidensaul provides in-depth coverage of many of his topics, including the historical details to which I am particularly partial. A reader well informed on current conservation issues may find much of the material familiar. But Weidensaul tackles so many topics—alien species, global warming, fire management, water use in the west, California condors, etc.—and treats many of them so thoroughly that almost everyone will find something new. Despite certainly not being written for the purpose, *Return to Wild America* would make an excellent textbook for introductory courses on environmental problems.

Everyone will have their own favorite sections. As one still haunted by the September 1914 death of the last passenger pigeon, once the world's most numerous bird, I especially appreciate Weidensaul's descriptions of biological abundance. His portrait of Florida's Dry Tortugas tern colonies brought back memories of my trip there in 1972. The streams of sooties and noddies illuminated by a low-hanging sun comprise a tableau that will be in my mind forever, even though I have since seen larger numbers in the South Pacific. Today, however, those colonies see probably fewer terns and definitely more tourists.

I am also indebted to the author for introducing me to a place I had never heard of before, the dry grasslands of Chihuahua called La Soledad. Weidensaul writes: "We rode out into the heart of the largest of the [prairie] dog towns…. As individuals, prairie dogs (which are, of course, actually large colonial ground squirrels) are immensely appealing…. But prairie dogs aren't about individuals; they're about numbers. And here at La Soledad, in the last place on the planet where they exist in truly enormous numbers, the scale simply overwhelms you." I have never been overwhelmed by prairie dogs, but it would appear to be a valuable experience.

True to his predecessors, Weidensaul ends his book on the Bering Sea's Pribilof Islands, home to hundreds of thousands of fur seals and some 4 million alcids, cormorants, gulls, and assorted other seabirds. But the biological richness of these remote bits of land is jeopardized by a host of factors, either definitely or possibly anthropogenic: overfishing, colonization by rats, increased water temperatures, historical whaling, and pollution. They are symptomatic of what prevails continentwide, for Weidensaul reports that most of what Peterson and Fisher saw in 1953 is still here, although bombarded by an ever-increasing array of threats. He discovered, to his surprise, "a far more optimistic picture emerging…than I ever expected." He cautiously concludes that with "action and vigilance," the United



Last stop. Like his predecessors, Weidensaul caps his account with a visit to the seabird and seal colonies of Alaska's Pribilof Islands [drawing by Roger Tory Peterson, from (*1*)].

CREDIT: ROGER TORY PETERSON, FROM PETERSON AND FISHER'S *WILD AMERICA*/COURTESY HOUGHTON MIFFLIN

The reviewer is the author of *A Natural History of the Chicago Region*. E-mail: joelgreenberg@earthlink.net

States can, if it chooses, "bind up the tattered edges of the continent's wild mantle and start to make right some of the mistakes of the past." I only hope this is true.

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10.1126/science.1120241

BEHAVIORAL ECOLOGY

Crowing About Culture

Joanna Dally

If asked the animal with which we share the most in common, we might be forgiven for naming one of the great apes. At first glance, our close evolutionary relationship with nonhuman primates would appear to make them the obvious choice. But John Marzluff and Tony Angell beg to differ. Fervent enthusiasts of all things corvid, they argue that "to know the crow is to know ourselves." Their *In the Company of Crows and Ravens* offers a general audience intriguing and inspiring insights into the dynamic relationship that has long persisted between corvids and humans. Using a rich tapestry of folklore and science, Marzluff and Angell provide a comprehensive account of the impact of crows on human culture throughout the ages. Their discussion moves from the earliest depictions of crow and man (in the cave paintings of Lascaux) to the more recent and ever-evolving relation between the two and the problems that this association often brings forth.

In the Company of Crows and Ravens
by John M. Marzluff and Tony Angell

Yale University Press, New Haven, CT, 2005. 404 pp. $30, £18.95. ISBN 0-300-10076-0.

Marzluff (a wildlife scientist at the University of Washington) and Angell (an artist and writer from the San Juan Islands of Washington) make the bidirectional nature of the corvid-human relationship the central theme of their book. They claim that the relationship represents an example of cultural coevolution. Although there is currently an argument for the convergent evolution of intelligence in corvids and great apes (*1*), the idea of cultural coevolution is a novel one. Indeed, culture and its possible existence in animals other than ourselves are contentious issues within the scientific community, not least because the word itself is loaded with uniquely human connotations. However, Marzluff and Angell use the term in its very broadest sense, arguing that "culture is a basic biological attribute of long-lived, social animals." That is, the authors define culture as representative of a socially transmitted behavioral pattern and cultural evolution as a change in any such behavior.

The authors describe a number of different behaviors that they deem indicative of culture in corvids. The most persuasive of these is the propensity for New Caledonian crows to use different types of extractive foraging tools depending on the geographic area in which they live (suggesting cultural transmission of tool use). Yet, although Marzluff and Angell marshal evidence for the existence of culture in corvids as well as humans, I cannot help but feel that their reasoning for the phenomenon of cultural coevolution falls short of compelling. For example, they describe how an increase in agricultural production precipitated a "cultural revolution" in crows as they began to exhibit a preference for feeding on cultivated crops. Consequently, farmers began to hunt and harass these birds, which, in turn, led crows to shift feeding sites toward unprotected crops or urban areas and to use more protected nest sites. Yet, even if cultural evolution is taken to mean only a change in a behavioral pattern as a result of social learning, this example seems to fall somewhat short of the benchmark. Shifts in crow populations toward or away from agricultural land need only have resulted from individual learning, a possibility in a species renowned for its inquisitiveness, boldness, and ingenuity that should not be overlooked. Admittedly, the authors do mention the possibility of trial-and-error learning rather than cultural change, but that fleeting discussion is perhaps indicative of their propensity to interpret experimental findings and anecdotal observations in a cognitively rich fashion.

Surprisingly, for such a comprehensive text, a few areas of corvid research are notably absent, including some aspects of memory. The authors discuss the ability of birds to remember where they have previously hidden items—the star of this mnemonic feat being the Clark's nutcracker, which can remember the location of cache sites for up to nine months—and they note the need of crows to protect food that has been hidden from potentially thieving competitors. However, Marzluff and Angell neglect to discuss the fascinating insights into crow cognition produced by investigations into the tactics birds use to protect their caches. Most notably, Nathan Emery and Nicola Clayton demonstrated that scrub-jays only take steps to prevent future thefts from caches after having been thieves themselves, the birds seemingly projecting their own experience of thievery onto competitors (*2*). Moreover, a corvid's memory for a caching event is not bound only to a recollection of where an item was hidden—far from it. At least one species, the western scrub-jay, is also able to mentally travel backward through time to remember what they hid where and when (*3*), and Clark's nutcrackers can remember the size of each item they have cached (*4*).

Crow craving corn.

CREDIT: TONY ANGELL/COURTESY YALE UNIVERSITY PRESS

The great strength of *In the Company of Crows and Ravens* lies in the obvious and inescapable passion that Marzluff and Angell have for corvids. One cannot help but be carried away by the authors' enthusiastic and engaging style. Their prose is supplemented by Angell's wonderful drawings and scratchboard illustrations. The authors state that one of their aims was to encourage people to watch and wonder at crows, and here they have been extremely successful. Indeed, if asked again with which animal we humans have the most in common, readers of the book might well be tempted to choose one of these remarkable birds.

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10.1126/science.1121307

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The Way Ahead™

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Following the Light: Opening Doors to Science in Tunisia

Zohra Ben Lakhdar

When I was young, everyone around me said that science was a field for men to pursue and that it was too difficult for women. They said there was a fundamental intellectual difference between men and women, and between girls and boys. The assumed role of women in society was to take care of the family. I did not accept these notions. I liked mathematics and all of the sciences, especially physics. It became my goal to show, through my own example, that I could do science as well as men could.

I attended primary school in the 1950s in the cities of Mahdia and Jemmal, where the highest level of education available to girls resulted in a "certificat d'études primaries." This is like a high school diploma, except that it marks the completion of primary school only. I know of no other girl, besides myself, who was in school with me at the time who even received that "diploma." There were few girls in the primary school I attended—less than about 25 in the first year—and only 6 of us made it all the way through to complete our education. Nobody even thought about going to secondary school, which required traveling 25 kilometers to the nearest big city, Sousse. With no buses or cars around, this was a very long and difficult trip. Most girls went just to primary school for a few years, and then got married, usually at around the age of 15 years.

> The Editors hope you have enjoyed this year's Global Voices of Science essay series celebrating 125 years of *Science*. The voices of the international community of scientists have an enormous amount to offer, and it has been our privilege to feature them in this year's anniversary events. We have learned much from their unique perspectives.
>
> Series editor, Ivan Amato

Marriage was by far our primary concern. That was what society expected of us. Our lives couldn't have been farther removed from science and technology. And that was true not only for girls. There were no Tunisian engineers, professors, or doctors in the country at all during that period. All of these professionals were French. One early source of inspiration for me was the importance my parents placed on the value of education. But even my father used to assume that his boys were the ones who could succeed in technical areas. He wanted his sons to be engineers. "Power is with science, and with people who are good in mathematics," he told them. Boys had power and opportunity by nature. I could see that women would have to earn their place in a man's world. This only fueled my desire to achieve the same scientific education and status as men and to open ways for other women to do the same.

There were good reasons to work toward this goal. As a child, I was dazzled by the power of science. I witnessed amazing feats, some of which took place in my own home. A French surgeon had saved my mother's life by performing open-heart surgery on her. The contraceptive pill was providing women with the power to decide when to have children and take on the rigors of raising a family. Men alone would no longer be the only ones with those powers. I witnessed the establishment of the industrial production of chicken: food for everyone! "Yahya el Elm," said my mother each time I tried to explain these things to her. That's essentially a tribute that means, "science be praised."

In 1956, when Tunisia gained its independence from France, women were granted equal rights with men under the law, and education became a primary issue in governmental politics. My family moved to Tunis, the nation's capital, a city now with a population of more than 3 million. There, I succeeded in entering secondary school. I spent 6 years in the best school for French and Arabic studies for women. I earned my baccalaureate (first part) with the best

CREDIT: MICHELINE PELLETIER/GAMMA

Zohra Ben Lakhdar
Tunisia

It has been almost 30 years since Zohra Ben Lakhdar received her appointment as a professor of physics at Tunis El Manar University in Tunisia. Now director of the Department of Physics' Laboratory of Atomic-Molecular Spectroscopy and Applications, she does both theoretical studies of the spectral properties of matter and applied research and development in several areas, including optics-based pollution monitoring. From her primary-school days onward, Lakhdar has had to battle political, social, and cultural obstacles as she muscled forward toward her lifelong goal of becoming an active and productive member of the global scientific community. After earning her Ph.D. in atomic spectroscopy from the University of Paris VI in 1978, she turned down offers to work in relatively luxurious conditions overseas and instead returned to Tunis University, where she has remained ever since. She has authored numerous papers and textbook chapters, advised and mentored many students, and was a founding member of the Tunisian Optical Society. In 1994, she was elected to the Islamic Academy of Sciences and since 2001 has been an associate member of the Abdus Salam International Centre for Theoretical Physics (ICTP) in Trieste, Italy. She also has organized and/or chaired international conferences and workshops in laser physics and related fields. Earlier this year, she was honored by being named a winner of the 2005 L'OREAL-UNESCO Award for Women In Science.

All essays and interactive features appearing in this series can be found online at www.sciencemag.org/sciext/globalvoices/

results at the national level. Unfortunately, though, this school had little to offer in the sciences. If I was going to become a scientist, I would have to take extraordinary steps.

Joining the Boy's Club

One important step that I took was to prepare for my baccalaureate (second part) in mathematics at Sadiki College, the best men's school in Tunis and one that was known particularly for its strengths in physics and mathematics. Nobody advised me to go there, though many were quick to tell me it would be too difficult for a woman. Yet, because of my strong academic record and because women now had equal rights with men under the law, girls also could gain access to Sadiki. Even so, other than myself, only one other woman attended the school. My parents were proud of my grades, which were very good, and I earned my degree in mathematics in 1963.

This was a degree that opened doors for me. With it, I could enroll in the Tunis University faculty of medicine, which had just been established, or in the faculty of sciences, which then was just 3 years old—the same age as the almost new university itself. I enrolled in the faculty of sciences and, because I had done well in mathematics, was awarded a grant from the government to pay for my courses. But I preferred physics, and I did my best to convince the government to let me use the grant to study physics. Of course, both fields are closely connected with one another.

This was a good time to be in higher education in Tunisia. To encourage students, the government offered each one a fellowship. This emphasis on education was still so new in the 1960s, however, that there weren't yet many students at the university level, particularly in physics or mathematics courses. Other than the two Tunisian staff members with Ph.D.'s, all of the professors were French. The country was proud of its first university students, especially of its science students. In 1963, there were only about 200 students in the science faculty, and only five of us were women.

Most of the students planned on becoming secondary school teachers. This was crucial for the country, as one of its goals was to build a self-sustaining educational system that, in time, could produce scientists and engineers. When it came time for me to graduate, however, I wanted to continue my scientific training. As it turned out, an opportunity arose for me to do so.

At the end of each academic year, in June, a professor came from France to supervise exams and to validate our diplomas. Each year, the Tunisian government awarded fellowships to the best three to five students to continue their studies at universities in France. In some cases, top students could use their fellowship to pursue basic research in France.

In 1967, I was selected by the university for the opportunity to go to France to study for a Diploma of Further Studies (Diplôma d'études approfondies, DEA). That year, the professor who came from France to validate our diplomas happened to be from the Université Pierre et Marie Curie, Jussieu (Paris VI), where he was a director of the Atomic Spectroscopy Laboratory (LSA), which was part of the physics department's Laboratory of Research (DRP). As it turns out, when I went to Paris, I ended up studying atomic spectroscopy at the LSA. I was close to the Sorbonne, the Collège de France, and the Ecole Normale Supérieure! For me, it was another world. I was in the world of atoms—the building blocks of matter—and of stars, and of cells! I was in a world of scientists! It was a world I hoped more Tunisian girls would now think was within their reach.

Pollution patrol. The author uses laser-induced fluorescence analysis to detect pollutants in plant tissue.

Each Tuesday, I went to the Collège de France and sat in on the quantum mechanics courses taught by Claude Cohen-Tannoudji. He led us into the atomic world step by step. He helped me to develop a deep love for the physics of atoms and to appreciate the simple beauty of this realm. Through him, I was exposed to the research of the Kastler-Brossel Laboratory where Alfred Kastler did much of the work on the interactions of light and atoms that led to the development of the laser and for which he earned the Nobel Prize in 1966. It was exciting to be so close to such momentous places and events in science.

At the same time, I was struck by a curious irony surrounding the technological feats of the era. For example, during the Apollo 11 mission in 1969, the entire world watched on television screens as Neil Armstrong became the first person to walk on the moon. Yet no one had ever photographed an atom. An image of an atom, free and in a stationary position, would be in hand only 20 years later. I found it amazing that exploring the world of atoms was in some ways more difficult than setting foot on the moon!

By 1971, I had earned my next degree. My thesis work focused on using spectroscopy and spectral analysis to deduce the potential of different atoms to interact with one another. All of my subsequent scientific work has emerged from this training. I am fascinated by what makes different substances take on different shapes and how substances undergo phase transformations.

I brought these experiences and my expanded knowledge back to Tunisia where I became only the second Tunisian woman to work as an assistant on the staff of the science faculty. I spent 3 years in this position, but I was unable to conduct my own research. For that, I needed a doctorate. So I returned to France, to the University of Paris VI. Following a 4-year period of study, during which I finally earned my "Doctorat d'Etat," my then-new husband, also a doctor of physics, and I both received offers to stay in France and build our careers there. That was tempting for both of us, but we chose to return to Tunisia to help plant scientific seeds, where we knew there were too few.

In 1978, I again found myself on the staff of the science faculty at Tunis University. This time, I joined as a professor of physics. I began working toward having my own research laboratory, so that I could offer my students the same opportunities that I had in France. It marked a new phase of my career.

It was a very big step for me, but each subsequent step—getting computers, software, and additional training, for example—has posed new challenges. It took 10 years for me to publish my first paper. Now my work includes theoretical studies of optical phenomena related to atoms and molecules, as well as the detection of air pollutants, using the technique known as tunable diode laser absorption spectroscopy (TDLAS); of water pollutants, using laser-induced breakdown spectroscopy (LIBS); and of pollutants in plant tissues, using laser-induced fluorescence (LIF).

Lighting the Way

Even as I had been taking these steps to show that women in Tunisia could become scientists if they wanted to, the mind-set of

CREDIT: MICHELINE PELLETIER/GAMMA

the population was hardly changing. For most people, even those highly placed in society, scientific expertise was something that belonged to Western countries. As a result, there was no scientific environment, no ambition, no motivation, and no political backing for research. Most women at the time considered it their main role to have a family and to raise children. Thinking about going abroad for research training or for postdoctoral work, ambitions that I had become accustomed to considering, were utterly foreign notions for most.

Of course, I had to work within certain constraints. Because we have not had many facilities or expensive instruments in Tunisia, for example, much of my scientific work has focused on theoretical studies of molecular interactions. Among the specific areas I have investigated are how laser-like effects and other optical phenomena could manifest in the rarefied matter found in various environments in space. I have been particularly interested in atomic states, molecules, and other forms of matter that cannot be replicated in the laboratory. Only by calculating the spectrum emitted by such entities is it possible to fully analyze the light that comes into our telescopes and spectrometers.

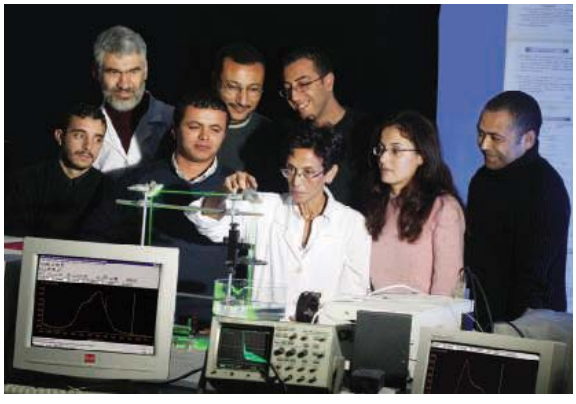
Even though I have been drawn to many arcane subjects in physics, including atomic spectroscopy, I have developed an interest in applying science in practical ways. I have seen how climate and lack of resources can thwart the aspiration of millions and make life very difficult. One project I worked on in recent years was the development of TDLAS methods for measuring trace pollutant gases in the atmosphere, including the methane that Tunisian industries emit.

In time, my dream is to develop technologies that would make it possible to convert deserts into arable land, transform sea water into potable water, reduce the ever more oppressive heat in our countries, and find ways to increase the amount of rain in dry areas. I also have a few more mundane visions. I would like to be able to explain why straight hair becomes curly with humidity, to find the molecular mechanism underlying that transformation, and to develop a product that can transform curly hair into straight hair. Even though so many women and girls throughout Africa have curly hair, many of them want straight hair, and they often spend a lot of time and money on this pursuit. Perhaps the most important thing I can do, however, is help to make it easier for those in my country and in other African nations to join the scientific community.

Along these lines, one of my dreams is to establish in Tunisia an international scientific center of optics and photonics (and physics education) where African researchers could easily come to study and train. My inspiration for this goal derives from the Nobel Laureate Abdus Salam, whom I greatly admire. In 1964, he created the International Centre for Theoretical Physics (ICTP) in Trieste, Italy, where researchers from developing countries can spend a few months, all expenses paid, working in a stimulating environment, taking courses on subjects of their choosing, meeting other scientists from around the world, and enjoying the luxury of a rich library. I have been an associate member of ICTP since 2001.

Since the days when I was one of the only girls and women in Tunisia with scientific aspirations, the number of women involved in the sciences has been increasing, particularly in the biological sciences. Part of the reason for this is that women have become more independent, making it possible for some of them to choose a career over marriage and motherhood. When I was a girl, my friends were getting married as teenagers. Now the average age of marriage for a woman is 27. Indeed, in Tunisia, both women and men now contribute equally to the development of the economy and society. As in more developed countries, these gains for women have come at a cost. Even as women have been making careers for the first time, they still bear most of the responsibility for attending to their family and raising their children.

My advice to young women scientists in my country is to persevere, to love work and to love to do good work, to be independent, to respect others but not submit to those who would stop you from achieving your goals, to be scientifically honest, and to embrace your ambitions, all the while respecting culture, responsibility to your family, and allegiance to your country.



Science huddle. Surrounded by colleagues, Zohra Ben Lakhdar analyzes water for pollutants using a technique called laser-induced breakdown spectroscopy.

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There is still a long way to go before science becomes an integral part of Tunisian society. Most women do not directly appreciate the importance of, say, physics, in the caring of the family, which remains the concern of every woman. That's why most women prefer to choose a job where the holidays are the same as they are for schoolchildren. Rather than taking jobs to further their careers, per se, women take them out of economic necessity to support their families. Moreover, their own careers usually are still less important to them than are the careers of their husbands. Even for those Tunisian women who do earn a place among professionals scientists, they often stop their research after obtaining a Ph.D. so that they can care for their children and family, while the men in the family continue on their career paths.

My advice to young women scientists in my country is to persevere, to love work and to love to do good work, to be independent, to respect others but not submit to those who would stop you from achieving your goals, to be scientifically honest, and to embrace your ambitions, all the while respecting culture, responsibility to your family, and allegiance to your country. Knowledge and know-how are the way of liberty and equality. Neither gender, nor religion, nor age will stand as a barrier to research in science. Yahya el Elm.

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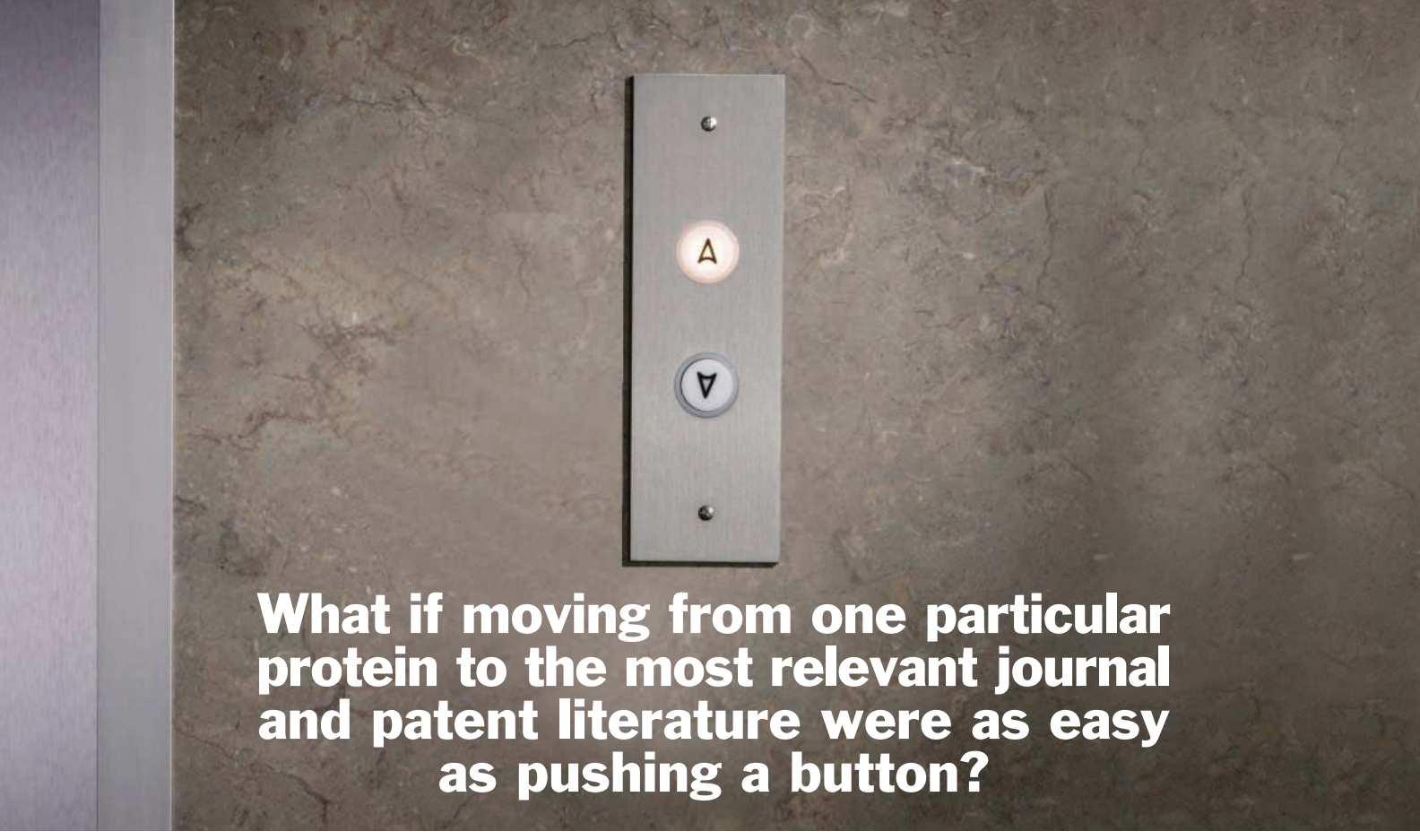

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PLANETARY SCIENCE

The Changing Picture of Volatiles and Climate on Mars

Bruce M. Jakosky, Robert M. Haberle, Raymond E. Arvidson

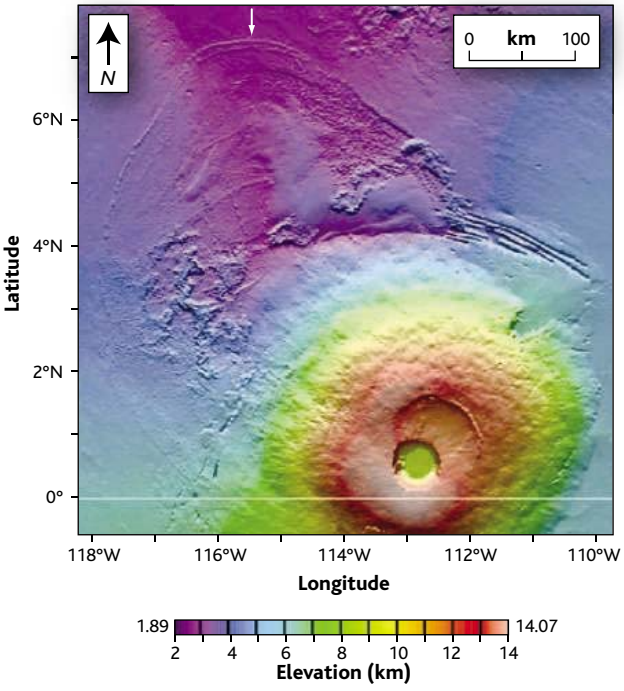
The behavior of water and other volatiles, such as carbon dioxide, is central to the questions that we are asking today about Mars' climate evolution and biological potential. Results from three spacecraft currently operating at Mars (Mars Global Surveyor, Mars Odyssey, and Mars Express), as well as from the continuing Spirit and Opportunity rover missions, have been changing our views in fundamental ways. This was evident, for example, at this year's Planet Mars II workshop (*1*) as well as in several recent papers. Among the most important issues discussed at the workshop and in the literature were seasonal cycles, obliquity variations, and climate changes over a time scale of 4 billion years.

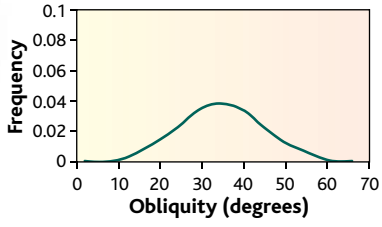
The standard wisdom since the 1970s about seasonal cycles on Mars has been that deposition and sublimation of CO_2 ice in the polar regions are governed by the local energy balance, that the seasonal CO_2 cycle controls the water cycle, and that every Mars year is basically similar to every other year. Recent observations suggest otherwise. The summertime residual CO_2 ice on the south polar cap is both thin (less than 10 m in thickness, equivalent to no more than about 3% of the amount of CO_2 that is in the global atmosphere) and discontinuous (*2*). Round and arcuate holes in the CO_2 ice cover ("Swiss cheese morphology") reveal underlying water ice that also is exposed elsewhere on the residual cap (*3*). The holes are enlarging each year, releasing CO_2 gas into the atmosphere. The morphology, stratigraphy, properties, sizes, and growth of the holes suggest multiple discrete events of deposition and erosion on time scales of decades to centuries (*2*). The changing exposure of south polar water ice may be reflected in the atmospheric water vapor content, which shows order-of-magnitude variations from one year to another during the southern summer.

Wintertime deposition of CO_2 frost is governed in part by atmospheric dynamical effects. Recent models show predominantly atmospheric condensation in some locations (including the location of the residual cap) and surface condensation in others (*4*). These processes redistribute energy from one location to another, so that the amount of frost deposited does not depend solely on the local energy balance. Thus, the CO_2 ice cover on the south polar cap may represent a balance between surface processes that tend to remove CO_2 ice and atmospheric processes that tend to redeposit it. These competing effects may produce substantial decades-long variations in the CO_2 frost behavior, with the seasonal water cycle responding. The behavior on longer time scales is governed by cumulative seasonal-cycle effects, so that extrapolation to longer time scales is extremely uncertain.

The tilt of the rotational axis of Mars also has a strong effect on seasonal cycles and climate, just as it does on Earth. Mars' axial obliquity varies on time scales of 100,000 and 1 million years. This has long been thought to affect climate through a changing polar cap energy balance that controls CO_2 ice-vapor equilibrium. During periods of higher obliquity, the amounts of water that move through the atmosphere should become enhanced as a result of increased summertime polar temperatures and water-ice sublimation. This latter process can change the distribution of ground ice in the top few meters, with the present boundary near ±60° latitude moving by as much as 10° to 20° in latitude (*5*). Morphology of the surface in the 30° to 50° latitude range suggests such an emplacement and removal of water ice (*6*).

Recent results suggest a much more dramatic climate effect caused by obliquity than previously believed. Statistical models of the obliquity variations now include chaotic variability on time scales longer than 10 million years. They show that the obliquity can range from 0° to as high as 80°, and that the most likely value is near 40° (*7*). Indeed, the current value of 25.2° seems low by comparison (see the figure). At values above about 40° to 50°, polar ice temperatures rise enough that outright melting of water ice would occur (*8*). At intermediate values, large amounts of water ice can sublimate into the atmosphere and condense onto the surface at lower latitudes. On the order of





Tilting toward a wetter Mars. (Left) Topographic map of Pavonis Mons volcano, showing ridges to the northeast of the volcano interpreted as glacial in origin. [Adapted from (*10*)] **(Right)** Statistical distribution of obliquity values for Mars over the past 250 million years, showing that today's value of 25.2° is significantly lower than typical [Adapted from (*7*)]

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hundreds to thousands of meters of ice can be deposited in some locations (*9*).

Recent geological mapping shows features at low latitudes best interpreted as glacial in origin (see the figure). Features occur in isolated low-latitude locations such as the flanks of the Tharsis volcanoes that are reminiscent of moraines, knobs formed as a residual similar to terrestrial water-ice sublimation hills, and flow-line morphology (*10*). Remarkably, these are the same locations for which dynamical models show preferential deposition of water ice (*9*).

Thus, the geological evidence supports the dramatic climate changes that would be induced by the changing obliquity. Indeed, large changes in the climate appear to be the natural consequence of the temporal oscillations of the system.

Surface features and geomorphology can also tell us much about the ancient climate. The occurrence of valley networks on the oldest surfaces and high erosion rates inferred from crater degradation and removal have long argued for a warmer and wetter environment on Mars earlier than about 3.7 billion years ago. The Sun was 30% less luminous then than it is today, increasing the greenhouse warming required to raise temperatures enough to allow liquid water. A thick CO_2 greenhouse atmosphere would have saturated at temperatures that were still too low, making a martian greenhouse problematic (*11*), and the radiative effects of dust or clouds may not have alleviated this problem (*12*). Impacts at the end of planetary formation may have mobilized water for brief periods, producing rainfall that might have formed the valley networks without requiring a sustained greenhouse (*13*). However, the timing of large impacts, the long tail in the decline of moderate-sized impacts and their climatic effects, and the requisite thickness of the atmosphere still need to be better understood.

Recent spacecraft results provide important new constraints on the history of liquid water. The Meridiani landing site for Opportunity has sulfate-rich deposits that require liquid water to have been present for sufficiently long times to have had significant geochemical effects (*14*). Mapping of these deposits from orbit (*15*, *16*) shows that they occur as regional rather than local deposits. Thus, the conditions allowing liquid water likely were produced by global rather than local conditions. In addition, clay minerals that are indicative of chemical weathering in the presence of liquid water occur only on the ancient surfaces (*16*).

These new results appear to have required the prolonged occurrence of liquid water during the early epochs. One possible mechanism is a relaxation of the constraints imposed by the faint young Sun (*17*). The early Sun would have been more luminous if it had been even slightly more massive; subsequent mass loss would have brought the Sun to its current mass. Allowable values of the early Sun's luminosity require less greenhouse warming, and a CO_2 greenhouse atmosphere is plausible. The measurements are few and the uncertainties large, however (*18*). And, if the faint young Sun problem is mitigated, the role of impacts in also mobilizing water is unclear.

The changes in our understanding of martian history and implications for climate and volatile evolution are not just minor tweaking of existing hypotheses; rather, they are changing our view of what the important processes have been. Admittedly, much uncertainty still surrounds the nature of the earliest climate and of the processes responsible for controlling it are still very uncertain. On the obliquity and the seasonal time scales, though, the evidence is both compelling and dramatic. As the history of liquid water is written in Mars' geological history, these new results, when properly digested, will be important for deciphering Mars' biological potential.

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GEOPHYSICS

The Ghost of an Earthquake

William C. Hammond

Unlike the shadowy remains of departed souls, past earthquakes can leave traces that are detectable today with modern geophysical instrumentation. If large enough, a seismic event in the upper crust (less than about 15-km depth) can change the state of stress in the Earth to mantle depths (depths greater than about 30 km. These stresses subsequently relax over time scales of weeks to decades. The length of time needed to fully equilibrate depends on the material properties of the lower crust and upper mantle, and the size and style of the earthquake. On page 1473 of this issue, Gourmelen and Amelung (*1*) report evidence that relaxation following large earthquakes in the early to mid-20th century is presently observable in central Nevada. Because it is transitory, the presence of this signal has profound implications for our interpretation of geodetic data with respect to crustal deformation in the western United States.

Enhanced online at www.sciencemag.org/cgi/content/full/310/5753/1440

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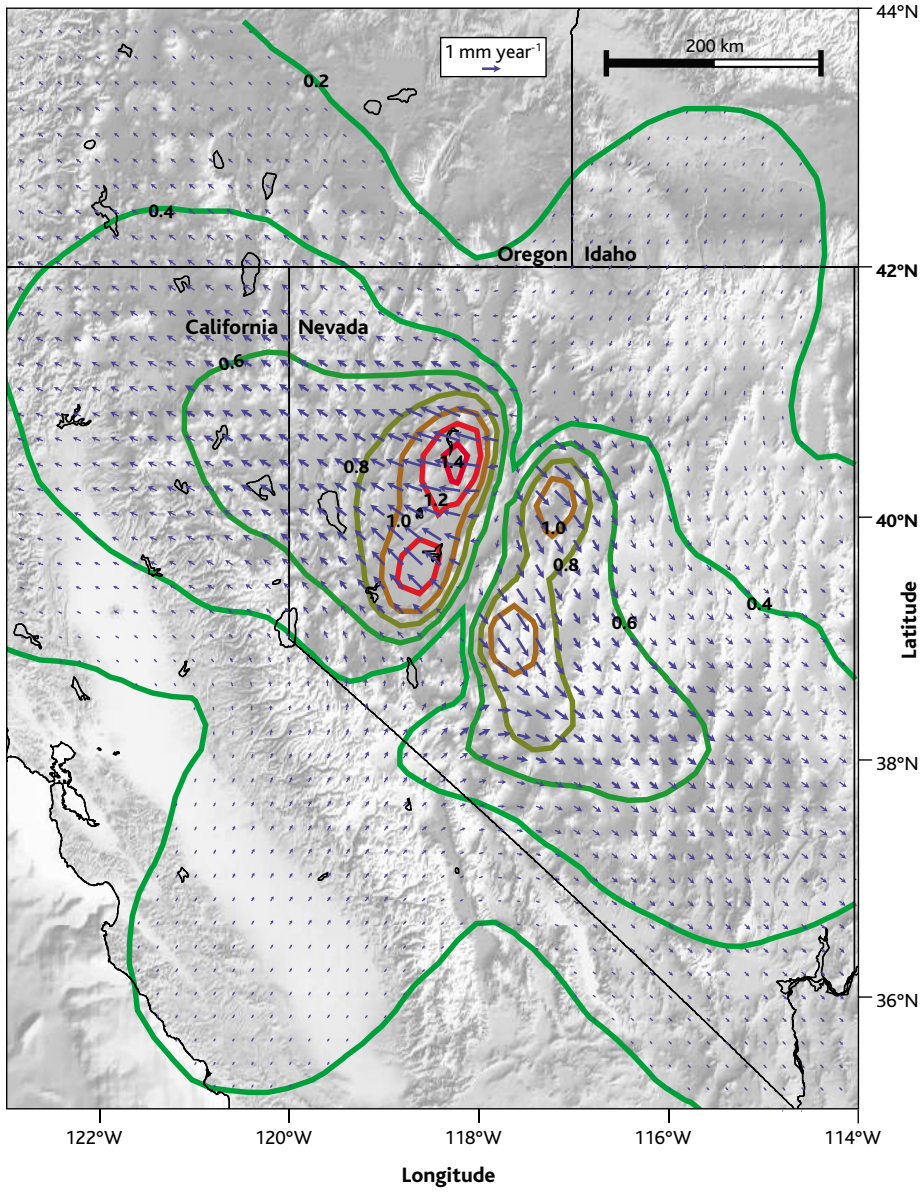
When subjected to loads, Earth's deep layers are thought to behave viscoelastically. Viscoelastic materials exhibit a component of viscous flow in their response to stress, in addition to an instantaneous (elastic) deformation. Therefore the response to an instantaneous stress change is drawn out in time, and in the specific case of Maxwell viscoelasticity used by Gourmelen and Amelung (*1*), decreases with time toward zero. Loads of sufficient size include sudden stress changes that occur in earthquakes (*2*), or the more gradual and/or time-variable loading owing to removal of continental ice sheets (*3*), or draining of large Pleistocene lakes (*4*, *5*).

Identifying and correcting for postseismic effects may be necessary when geodetic measurements, such as those that are frequently made with the Global Positioning System (GPS), are used to map the slow, inexorable motion of tectonic blocks. Such measurements have helped show that the Basin and Range province of the interior western United States is a part of the wide (~1000 km) and diffuse plate boundary deformation zone that accommodates the relative motion between the Pacific and North American plates (*6*). In these regions, GPS measurements are used to quantify seismic hazard by estimating deformation rates near active faults. Thus, the existence of transient deformation features that are hundreds of kilometers wide, with deformation rates up to several millimeters per

year, is of considerable interest. Because transient effects do not represent the time-invariant fault loading that is typically assumed in seismic hazard assessment studies, these effects must be estimated and removed. The figure shows the predicted magnitude and direction of horizontal velocity that the 20th-century earthquakes in central Nevada could have (*7*), according to the Gourmelen and Amelung model (*1*) and relaxation theory (*8*). The maximum predicted change in velocity is ~2.5 mm year^{-1} across the location of the Pleasant Valley 1915 rupture, an effect that is quite large when considering that the total relative motion across the western Great Basin is ~10 mm year^{-1}. However, distinguishing the horizontal relaxation from the background secular deformation of the Basin and Range is difficult because the two fields are superimposed.

The validity of the model and the correction that it implies are supported by a comparison between paleoseismically and geodetically inferred slip rates on the faults that activated during the 20th-century Nevada earthquakes. These slip rates independently obtained by geodesy and paleoseismic techniques disagree to a level well outside the uncertainties in those techniques (*9*). Theoretically, however, slip rates obtained by geologic means (i.e., through estimating the size, style, and history of markers offset by earthquake rupture) should be in rough agreement with rates inferred by geodetic measurement. This explicitly assumes that geodesy measures crustal strain accumulation that is followed by episodic seismic release in earthquakes, and that the slip rate is approximately constant over time. In reality, both of these assumptions can be in error. In some cases, fault slip rates have been inferred to change over time [see, for example, (*10*, *11*)]. Alternatively, as in the case of central Nevada, the geodetic field can be "contaminated" by viscoelastic aftereffects, giving the false impression of slip rate disagreement. Crucial to the plausibility of the relaxation model presented by Gourmelen and Amelung (*1*) is the fact that it explains this discrepancy extremely well by reducing the amount of the geodetic deformation attributable to the steady time-invariant motion of tectonic blocks.

The Gourmelen and Amelung results (*1*) contribute directly to a longstanding debate over the location of strength in the continental lithosphere. The Interferometric Synthetic Aperture Radar (InSAR) data, provide very high resolution images of surface changes over time. Using these data, they infer that the Basin and Range lower crust has higher viscosity than the upper mantle, a result that agrees with studies



Postseismic relaxation. Contours show the magnitude, and vectors (blue) show the horizontal velocity, of surface motion (in millimeters per year) from the model of postseismic relaxation that is consistent with InSAR and GPS data (*1*, *7*). In contrast to the vertical motion that is focused within ~100 km of the seismic events (*1*), the horizontal component extends many hundreds of kilometers from the epicenters, but is more difficult to distinguish from the background secular deformation. Velocity is with respect to the central Nevada seismic belt faults, which are located approximately between the lobes of rapid postseismic motion (*1*).

based on modeling geodetic data obtained after earthquakes, and Pleistocene lake unloading. However, it is in disagreement with other studies that suggest that the lower crust may be weaker than the mantle (*12*) owing to compositional differences (*13*), or the need to explain the presence of metamorphic core complexes (*14*). The vertical position of this strength is important because it determines the extent to which the geometry and orientation of crustal blocks control deformation of the North America plate. Strength residing in the uppermost crust implies lithospheric deformation controlled by friction on faults. However, if the strength resides primarily in deeper layers, then the crustal blocks delineated by surface faulting are reacting passively to stresses from below. Considerable uncertainty still exists over where the resistance to lithospheric deformation comes from, and is likely to be the subject of much future research.

A fascinating possibility discussed by Gourmelen and Amelung (*1*) is the evaluation of past earthquake magnitude based on the characteristics of the postseismic response. This prospect may be more tenu-

ous because of the analytical challenge involved in resolving the ambiguity between the offset from the earthquake and the viscoelastic structure. However, this approach adds to the small number of methods available for directly linking seismic and geodetic observations with the goal of understanding the entire earthquake cycle. This work represents a step toward understanding the longest periods of the postseismic response as an integral part of the earthquake process. Given the importance and difficulties of evaluating total earthquake magnitude in events like the 2004/2005 Sumatra great earthquakes, and because much of the energy release and stress transfer from such earthquakes can occur months to decades after the event, any new constraints are welcome. In this way we put the ghosts of earthquakes to good use.

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IMMUNOLOGY

Tipping the Scales Toward More Effective Antibodies

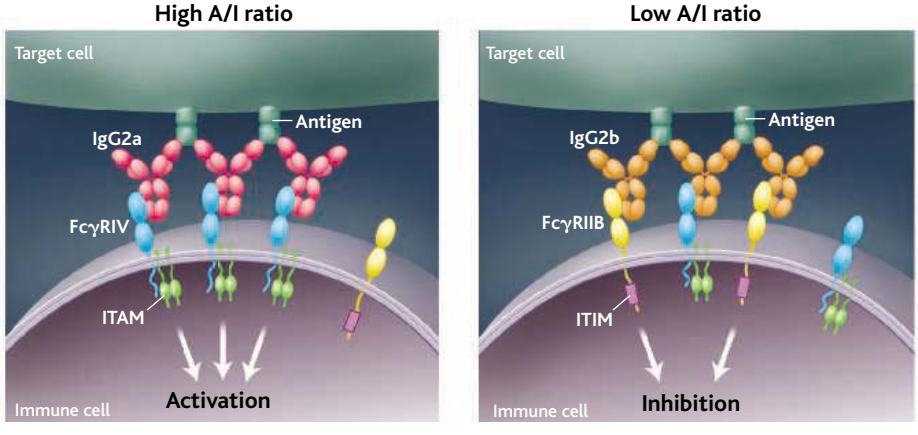
Jenny M. Woof

Antibodies are lifesavers par excellence. Not only do these vital proteins of the immune system armory protect millions of people across the globe after being elicited in vaccination programs, but also they serve increasingly as potent therapeutics in the clinic. Monoclonal antibodies, homogeneous antibody preparations specific for single antigens that have long been heralded as magic bullets, are finally fulfilling their promise. Indeed, monoclonal antibodies directed against targets such as cancer cells represent a major focus of the biopharmaceutical industry. But antibodies come in many different classes and subclasses, so how do we decide which category of antibody is most suitable for a particular clinical application? Mouse models of diseases can provide important insights, and a report by Nimmerjahn and Ravetch on page 1510 of this issue (*1*) raises the possibility of predicting the in vivo efficacy of individual immunoglobulin G (IgG) subclasses in treating particular cancers and infections.

IgG, the major immunoglobulin class in serum, exists as four structurally distinct subclasses in humans and mice. All IgG subclasses recognize antigens on foreign cells but have markedly different abilities to trigger immune mechanisms to eliminate these foreign targets. The latter processes rely on interaction of the Fc region of the antibody with effector molecules such as complement in the serum or Fc receptors (FcγRs) expressed on a variety of immune cells. FcγRs also come in different classes (*2*). In humans, there are three types: FcγRI, FcγRII, and FcγRIII. FcγRII is further subdivided into FcγRIIA, FcγRIIB, and FcγRIIC. Although the correspondence is not absolute, mice also have FcγRI, FcγRII, and FcγRIII, along with a recently discovered additional class, FcγRIV, which is absent in humans (*3*). Mice have only the FcγRIIB form of FcγRII.

In terms of function, FcγRs fall into two camps—those that activate a cellular response, and those that block it. Activatory receptors usually associate with a transmembrane signaling component that carries an immunoreceptor tyrosine-based activation motif (ITAM), or in some cases the activatory receptor possesses its own ITAM. In contrast, the single inhibitory receptor FcγRIIB carries a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM). When one or more FcγRs on an FcγR-positive cell simultaneously bind IgG presented in an aggregated form (e.g., on the surface of a foreign cell), cellular responses are triggered. Engagement of activatory receptors initiates intracellular kinase cascades, culminating in responses such as phagocytosis and release of inflammatory mediators. On the other hand, engagement of both activatory and inhibitory receptors by antigen results in recruitment of intracellular phosphatases that effectively terminate any activation. Because activatory and inhibitory receptors are frequently coexpressed on immune cells such as macrophages and monocytes, the final nature of the cellular response reflects a finely tuned balance between activatory and inhibitory signaling.

How can we better understand the rules



Activation versus inhibition. With a high activatory to inhibitory (A/I) ratio, IgG2a (red) antibody binds antigen (dark green) on a target cell surface and preferentially binds to activatory receptor FcγRIV (blue) rather than to inhibitory receptor FcγRIIB (yellow), and immune cell activation results. In contrast, an IgG2b antibody (orange), with a lower A/I ratio, will bind both FcγRIV and FcγRIIB and activation is dampened.

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of engagement of FcγRs so as to predict the cellular outcome? The situation is complicated by the fact that each IgG subclass displays different affinities for the various types of FcγRs. Nimmerjahn and Ravetch's starting point was to determine the affinities of each IgG subclass for soluble forms of different FcγRs, and then divide the affinity for the relevant activating receptor (either FcγRIII or FcγRIV, depending on IgG subclass) by that for the inhibitory receptor (FcγRIIB). This calculation yields an activatory to inhibitory (A/I) ratio. The A/I ratios of the mouse IgG subclasses differed markedly, with values of 69 for the IgG2a subclass, 7 for IgG2b, and 0.1 for IgG1. IgG3 showed no detectable binding to the receptors tested, so no A/I ratio was assigned.

They subsequently used two mouse model systems to test whether these A/I ratios could predict in vivo outcomes. In one model, IgG subclasses with identical tumor antigen specificity were assessed for the ability to clear lung metastases in mice injected with tumor cells. In the other, matched integrin-specific IgG subclasses were tested for performance in clearing integrin-bearing platelets. In both cases there was a very good correlation between high A/I ratio and biological efficacy. Further experiments with mice deficient in FcγRs or complement components, or in which the activatory FcγRIV was blocked by a specific monoclonal antibody, indicated that, in these antigen models at least, the in vivo activity of the antibodies depended on activatory FcγRs and not complement. Eliminating expression of the inhibitory receptor FcγRIIB in mice by genetic knockout had the greatest impact on the in vivo activity of the IgG subclasses with low A/I ratios. Finally, the investigators modulated the A/I ratios of the IgG subclasses by altering their glycosylation profiles. Consistent with other studies (*4*), antibodies deficient in fucose had increased affinities for the various FcγRs, and therefore had different A/I ratios. Again, a correlation between A/I ratio and efficacy was noted. For example, the A/I of IgG2b increased from 7 to 20 upon defucosylation, which translated into considerably enhanced in vivo activity. Overall, the tests show that the A/I ratio of a particular IgG subclass is a good predictor of efficacy in a rodent model. However, for general applicability, it might be desirable to adjust the A/I ratios by factoring in the expression levels of each Fc receptor.

How might this translate to the human system? Can we select IgG subclasses for optimal in vivo effect? It's not clear yet, but important differences between the mouse and human systems may foreshadow difficulties. First, there is no direct correspondence between mouse and human IgG subclasses. Second, unlike mice, humans possess activatory versions of FcγRII (FcγRIIA and C) that share the same IgG subclass binding affinities as the inhibitory FcγRIIB, thereby complicating A/I ratios. Third, receptor polymorphisms may obscure the picture. For example, an FcγRIIA polymorphism renders only some individuals able to bind human IgG2 by this receptor. Finally, in the human system, complement is suggested to contribute to antibody-mediated elimination of certain targets (*5*, *6*). However, for antibody therapeutics, the prospect of predicting suitable antibody isotypes is appealing, especially for companies eager to minimize development costs. One thing is certain: More and more monoclonal antibodies are headed for the clinic, and a means of accurately predicting antibody performance at an early stage is a goal worth pursuing.

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CELL BIOLOGY

Keeping Survivin Nimble at Centromeres in Mitosis

William C. Earnshaw

No one would expect an orchestra to perform Beethoven's ninth symphony without a conductor. It is therefore no surprise that when cells perform their most dramatic tour de force—division by mitosis—the process is carefully directed by a team of regulators. Like a conductor, these regulators activate key players in the mitotic program at precise times and locations within the cell. Some act globally. For example, activation of key cyclin-dependent kinases triggers the start of mitotic events throughout the cell, whereas activation of the ubiquitin-dependent proteolysis system by the anaphase-promoting complex/cyclosome similarly triggers the exit from mitosis. Other regulators, such as the chromosomal passenger kinase complex, link this temporal regulation with action at particular locations in the cell. They do this by moving from place to place to target critical cellular components and choreograph mitotic progression (*1*, *2*). On page 1499 in this issue, Vong *et al.* (*3*) report the surprising discovery that the mobility of one of the essential components of the passenger complex, survivin, is regulated by its modification with the protein ubiquitin.

Survivin has attracted considerable interest—and controversy—over its brief history. Because sequence motifs in the protein resemble those of baculovirus "inhibitor of apoptosis" proteins, it was originally linked to the regulation of cell death. However, in cells or in mice lacking survivin, the phenotypes most closely correlate with defects in mitosis (*4*, *5*). The link with mitosis was confirmed when it was realized that survivin is a member of the chromosomal passenger complex, proteins that move between chromosomes and the spindle midzone during cell division. The passenger complex also includes the aurora B protein kinase, inner centromere protein (INCENP), and borealin/Dasra-B (*6*, *7*) (see the figure). This association of proteins corrects attachment errors between chromosomes and the mitotic spindle, regulates the quality-control checkpoint that monitors those attachments, and ensures the proper completion of cytokinesis (*2*).

Perhaps the most interesting aspect of chromosomal passenger behavior is the way in which the complex moves from place to place during mitosis. The complex is present and functional at centromeres (that part of the chromosome to which the mitotic spindle fibers attach) during prometaphase, when chromosomes begin to bind to microtubules. However, it relocates to the central spindle at the onset of anaphase, when chromosomes migrate to opposite poles of the cell. The complex then moves to the equatorial cortex just before cleavage furrow assembly and cytokinesis ensues.

How does this chromosomal passenger complex move from site to site? Many proteins change their behavior when they are modified by phosphorylation, but Vong *et al.* reveal that modification with ubiquitin has a key role regulating survivin mobility (*3*). They show that in the cytosol of frog eggs,

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where components required for cell division during early embryonic development are stockpiled, survivin is associated both with a deubiquitinating enzyme known as xFAM, and a complex of three proteins—p97, NP14, and Ufd1—that together recruit ubiquitin ligases to target proteins (*8*).

Ubiquitin is best known for its role in protein degradation, but it has other functions as well. Ubiquitin can be attached to a target protein as a chain, with links via the lysine 48 (K48) residue of the ubiquitin. If the polyubiquitin chain is linked to the target protein via this residue, the modification acts as a signal for degradation of the target protein. On the other hand, linkage of ubiquitin proteins to each other via lysine 63 (K63) creates a "flag" that is important for regulating the behavior of the attached protein (*9*). Interestingly, in mitotic cells, ubiquitin with both K43 and K68 linkages is found on survivin.

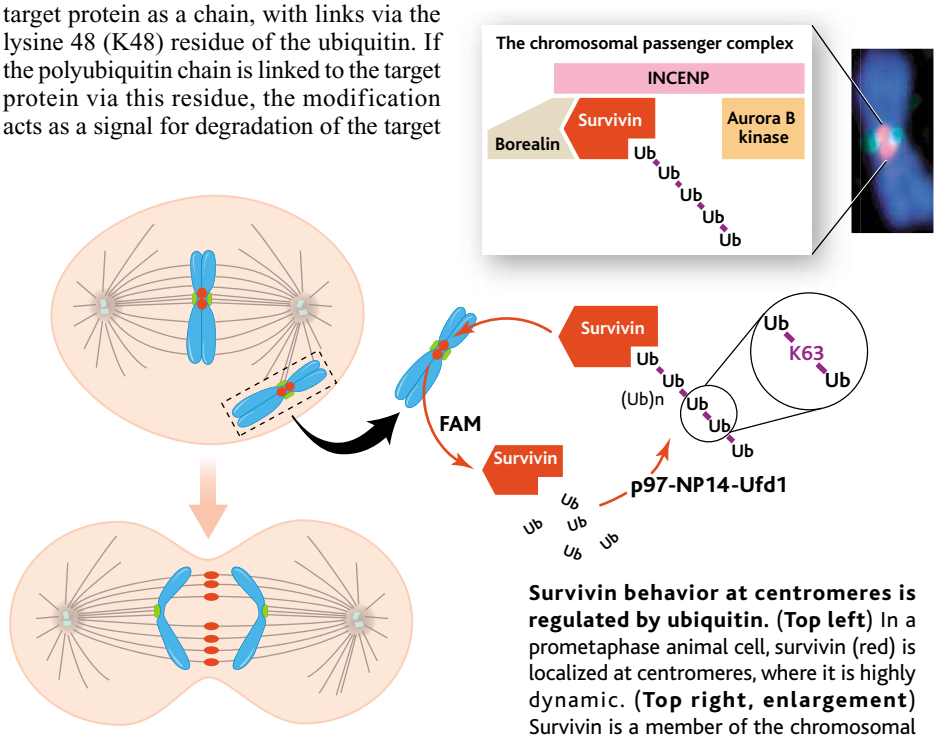
The protein complex of p97-NP14-Ufd1 acts as a chaperone that recruits ubiquitin ligases to target proteins. When expression of the Ufd1 subunit is reduced by RNA interference (RNAi), the amount of K63-linked polyubiquitin on survivin drops substantially, but the overall expression level of survivin does not change (*3*). This suggests that the K63-linked ubiquitin might be involved in regulating the behavior and function of survivin, rather than its stability. Indeed, in cells where Ufd1 expression is reduced by RNAi, survivin is unable to accumulate at centromeres, and cells have a hard time aligning their chromosomes on the spindle.

Depletion of hFAM from a human cell line by RNAi also has no effect on survivin expression, but it does cause mitotic abnormalities including misaligned chromosomes and lagging chromosomes at anaphase (*3*). Interestingly, although survivin depletion by RNAi causes a marked cytokinesis defect, reduction of FAM expression by RNAi does not. Thus, K63 ubiquitination of survivin is involved in regulating its function at centromeres, but not at the cleavage furrow. In keeping with this, although survivin behavior at centromeres is perturbed when FAM expression is reduced, its localization to the central mitotic spindle and midbody (the microtubule bundle that forms between the two separated daughter cell nuclei late in mitosis) appears to be unaffected.

FAM turns out to be required for survivin behavior at centromeres in a particularly interesting way. In FAM-depleted cells, the amount of survivin on the centromeres of chromosomes that are aligned on the mitotic spindle decreases, but survivin (and aurora B) on misaligned chromosomes increases, with the protein spilling over onto the chromosome arms. Studies using fluorescent probes to quantify movements of molecules inside living cells have shown that survivin localization at centromeres is dynamic (*10*, *11*). These dynamics are substantially (up to 50-fold) quenched after FAM expression is reduced (*3*).

Vong *et al.* (*3*) find that when the amount of K63-linked ubiquitin on survivin is too low, the protein cannot accumulate at centromeres, and chromosome segregation is perturbed. On the other hand, if too much K63-linked ubiquitin is associated with survivin, then the protein is stuck at centromeres and cannot move around normally. This also perturbs chromosome segregation. Together, these results reveal that the balance between K63-linked ubiquitination and deubiquitination plays a key role in regulating survivin function, apparently by regulating the mobility of the protein (see the figure). Why the inhibition of survivin dynamics at centromeres, caused by overaccumulation of K63-linked ubiquitin, impairs the ability of the chromosomal passenger complex to tinker with chromosome attachments to the spindle microtubules is not known. Nor is it clear how this in turn causes defects in chromosome alignment in the mitotic spindle and segregation when cells enter anaphase. Apparently, the correct balance between K63-linked ubiquitination promoted by p97-NP14-Ufd1, and deubiquitination by FAM, ensures that survivin spends just the right amount of time at centromeres to properly regulate chromosome-spindle attachments.

In addition to illustrating a new role for ubiquitin in controlling the behavior of a key mitotic regulator during mitosis, the Vong *et al.* study raises interesting questions for future research. As pointed out by the authors, there are myriads of deubiquitinating enzymes and their regulators, whose functions remain to be determined. Are we on the cusp of discovering a whole new role for ubiquitin in regulating the dynamic mobility of cellular proteins in mitosis and beyond?



Survivin behavior at centromeres is regulated by ubiquitin. (**Top left**) In a prometaphase animal cell, survivin (red) is localized at centromeres, where it is highly dynamic. (**Top right, enlargement**) Survivin is a member of the chromosomal passenger complex, which coordinates many key events during mitosis. The Aurora B kinase regulates microtubule attachment and checkpoint function at centromeres early in mitosis. (**Top right**) INCENP (pink fluorescence), which colocalizes with survivin in the inner centromere, is flanked by green fluorescent dots showing the points of microtubule attachment at kinetochores. (**Center**) Survivin targeting to centromeres requires the attachment of ubiquitin (Ub), a modification that is stimulated by the p97-NP14–Ufd1 complex. The dynamic behavior of survivin at centromeres requires the activity of the deubiquitinating enzyme FAM. (**Bottom left**) Later, during anaphase, when survivin transfers to the central mitotic spindle, its behavior no longer requires p97-NP14-Ufd1 and FAM.

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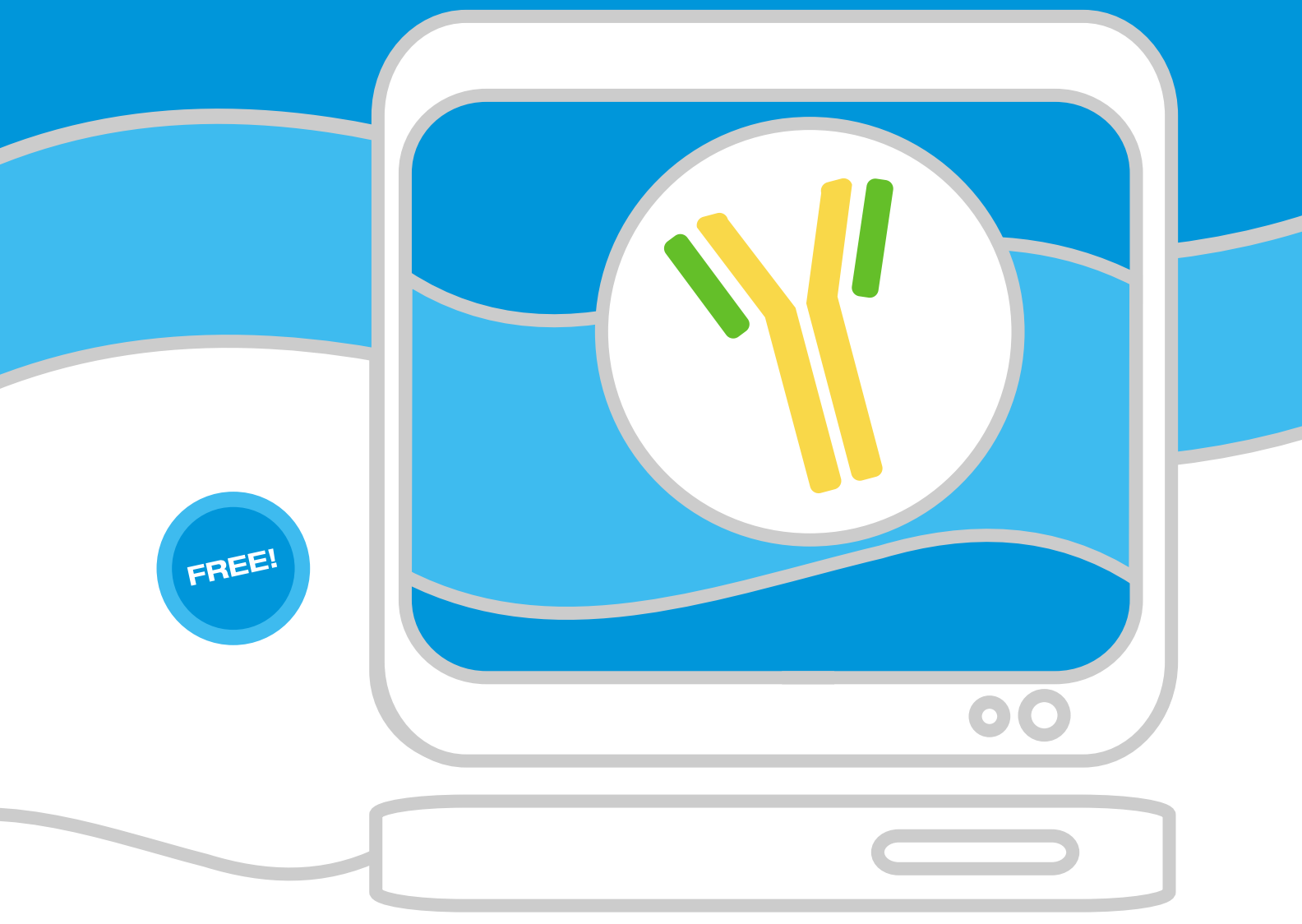

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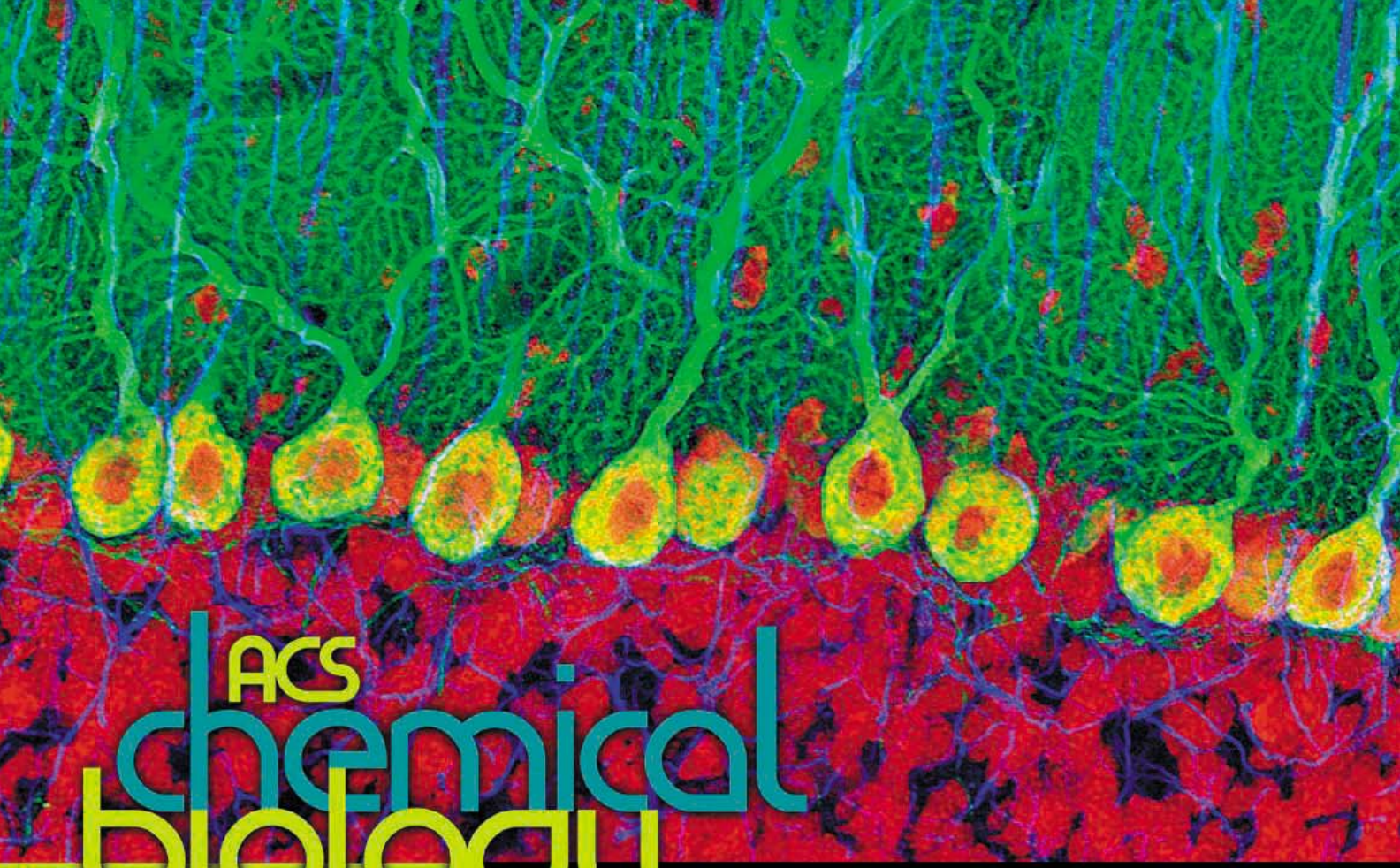

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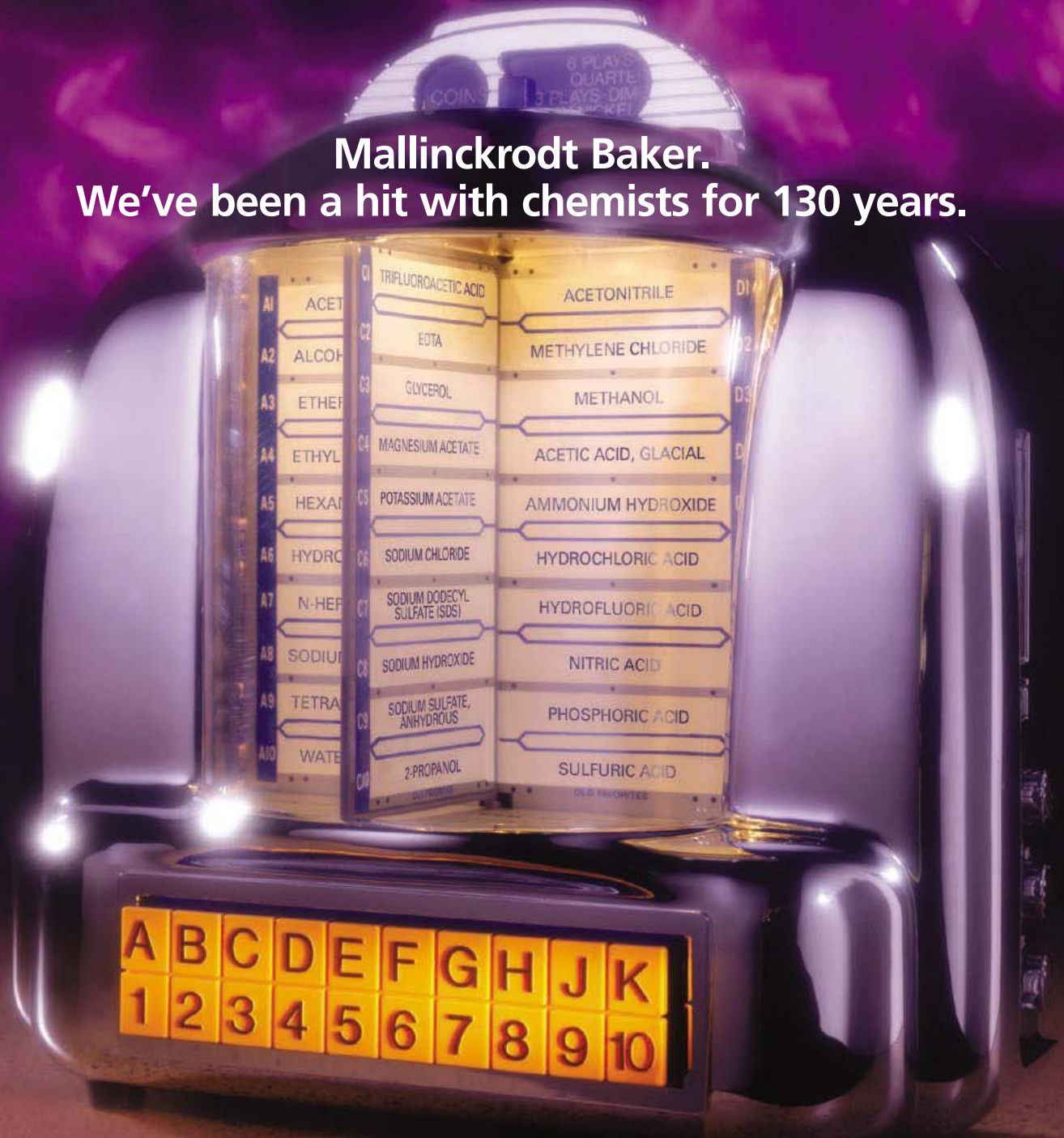

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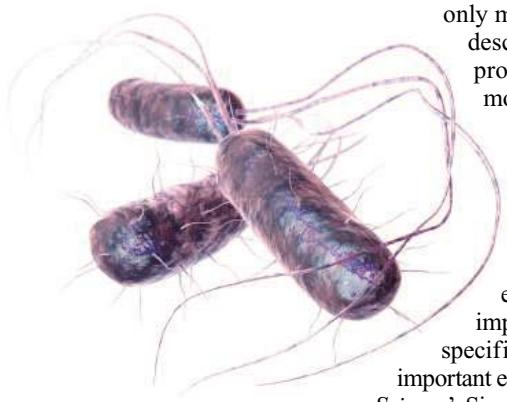
INTRODUCTION

Crossing the Bilayer

All cells, whether bacterial, plant, or animal, are enclosed by membranes, the basic components of which are lipid bilayers. The cell membrane ultimately acts as the defining principle of what constitutes a cell and what constitutes the rest of the world. Lipid bilayers are semipermeable: Small uncharged molecules can pass more or less freely from one side of the membrane to the other, but for charged species or macromolecules, such as proteins and DNA, the lipid bilayer is a major obstacle to diffusion. However, in real life, cells need to be able to transport proteins, DNA, and ions into and out of the cell, across the lipid bilayer. In this special issue, we look at the mechanisms used by cells to allow proteins, DNA, and ions to directly traverse a biological membrane.

Wickner and Schekman (p. 1452) describe how proteins can cross, or become integrated into, specific membranes. The endoplasmic reticulum membrane in eukaryotes and the plasma membrane in bacteria contain a proteinaceous pore—the translocon—that specifically promotes the translocation and integration of a multitude of signal-sequence–bearing membrane and secretory proteins into and through the membranes. Beyond these canonical systems that use the translocon, the authors mention translocation machineries and targeting strategies involved in import into different organelles, such as the mitochondria, chloroplasts, and peroxisomes, within cells and across kingdoms. Proteins are not the only macromolecules transferred across the membrane. Chen *et al*. (p. 1456) describe how bacteria allow DNA to traverse their membranes during the processes of conjugation and transformation and compare and contrast the molecular machineries involved in targeting and transport.



Regulating the internal composition of the cytosol is a key process in maintaining the chemistry of life, and two of the most fundamental components are the concentration and intracellular/extracellular balance of a variety of biologically important ions, including sodium, potassium, calcium, and chloride. Gouaux and MacKinnon (p. 1461) describe how ions get across membranes via transmembrane pumps and channels and explain the chemical and structural constraints involved. They describe the importance of gating within these structures, which allows for the very high specificity of transport observed and for the establishment and maintenance of important electrochemical gradients across cell membranes.

Science's Signal Transduction Knowledge Environment (STKE, stke.sciencemag.org) addresses how information is transmitted across cell membranes to contribute to cell signaling processes. When ions and proteins cross cell membranes, they may trigger intracellular signaling cascades. Hisatsune and Mikoshiba describe how in mammalian cells, the inositol phosphate receptor (the IP_3 receptor) and the calcium sensor (STIM) redistribute and cluster in response to changes in intracellular calcium and mediate calcium influx into the endoplasmic reticulum to refill intracellular calcium stores. Joliot describes how a class of cell-penetrating peptides can allow cells to communicate with one another, and Önfelt *et al*. describe how nanotubular membrane connections mediate intercellular communication. The Teaching Resource by Felsenfeld provides lecture materials describing how integrins transmit information from the extracellular matrix to the cytoskeleton.

How cells generate and maintain their internal structures and integrity depends in large part on the effectiveness of the membrane in keeping the inside in and the outside out. The mechanisms used in the transfer of ions and macromolecules across the cell membrane can thus be considered one of the defining principles of life, and our understanding of these processes is fundamental to our understanding of all other aspects of cellular and organismal physiology.

–STELLA M. HURTLEY

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Science

REVIEW

Protein Translocation Across Biological Membranes

William Wickner[1]* and Randy Schekman[2]*

Subcellular compartments have unique protein compositions, yet protein synthesis only occurs in the cytosol and in mitochondria and chloroplasts. How do proteins get where they need to go? The first steps are targeting to an organelle and efficient translocation across its limiting membrane. Given that most transport systems are exquisitely substrate specific, how are diverse protein sequences recognized for translocation? Are they translocated as linear polypeptide chains or after folding? During translocation, how are diverse amino acyl side chains accommodated? What are the proteins and the lipid environment that catalyze transport and couple it to energy? How is translocation coordinated with protein synthesis and folding, and how are partially translocated transmembrane proteins released into the lipid bilayer? We review here the marked progress of the past 35 years and salient questions for future work.

Emerging technologies have enabled progress in understanding protein translocation. In vitro protein synthesis led to the discovery that the mRNAs for secreted proteins are attached to membranes, whereas cytosolic proteins are made on free polysomes (*1*–*3*). The junction between endoplasmic reticulum (ER)–bound polysomes and the membrane is tight enough to exclude protease (*4*), and nascent chains are discharged directly into the lumen (*5*). Mouse myeloma mRNA was found to encode a polypeptide 1.5 kD larger than mature immunoglobulin light chain (*6*). The larger form was postulated to be a precursor with an N-terminal extension that specifies secretion, a research direction that culminated in the signal hypothesis (*7*). "Signal sequences" were deciphered for proteins that crossed a membrane, including the ER, mitochondria, chloroplast, and bacterial envelope. Armed with a signal sequence, mature domains merely had to be susceptible to unfolding in order to translocate (*8*, *9*). The signal sequences of each organelle have shared motifs of polarity and structure but no sequence conservation. For example, bacterial or ER export signals are basic at the N terminus, followed by a stretch of 8 to 14 apolar residues and a short cleavage motif that is recognized by a dedicated peptidase. Bacterial *sec* (secretion) genes, encoding proteins that support translocation, were identified either as suppressors of signal sequence mutants (*10*) or as temperature-sensitive mutants that failed to initiate secretion at the nonpermissive temperature (*11*). The yeast Sec61p (*12*) is homologous to bacterial SecY, establishing that the membrane-embedded portion of these translocons is conserved. In vitro translocation reactions were developed by adding isolated organelles to protein synthesis extracts for ER (*7*), mitochondria (*13*, *14*), chloroplasts (*15*), and bacterial plasma membrane (*16*, *17*). These in vitro systems were the basis for discovering energy requirements, determining whether translocation needed chaperones or ongoing protein synthesis, and initiating enzymological dissection of the process. In most cases, preproteins engage with chaperones or ribosomes, bind to a membrane receptor (which may also serve as a motor), transfer to a membrane-embedded translocon, and are released laterally into the lipid bilayer or completely cross the membrane with the aid of chaperones on the trans surface (Fig. 1A).

The Sec Translocase

Although ER and bacterial proteins share the same signal sequences, early studies suggested that their translocation mechanisms are very different. Bacterial translocation requires both adenosine 5′-triphosphate (ATP) and the membrane electrochemical potential, whereas ER translocation only needs nucleotides. Only short nascent preproteins can initiate translocation into canine ER (*18*). Signal recognition particle (SRP), a complex of 7*S* RNA and six polypeptides, coordinates translation and translocation (Fig. 1B) through binding to emerging signal sequences and slowing chain growth (*19*, *20*). Translocation resumes when the complex of polysome, nascent chain, and SRP reaches its ER-bound receptor (*21*–*23*). In contrast, preprotein translocation across the bacterial plasma membrane is not coupled to translation, either in vivo (*24*, *25*) or in vitro (*26*). Specific proteins provide the mechanistic basis for this dichotomy; some full-length preproteins that have left the ribosome bind to the SecB chaperone and then engage the SecA protein as their membrane receptor (Fig. 2) (*27*). SecA is activated to bind and hydrolyze ATP for powering translocation by its associations with the signal sequence and mature domain of a preprotein and with the SecYEG translocon, the membrane receptor for SecA (*27*, *28*). By the mid-1980s, it appeared that ER and bacterial translocation were fundamentally different, although this distinction soon blurred.

The membrane-embedded proteins needed for translocation were isolated by solubilizing membranes in detergent, fractionating the mixed micellar extracts, and assaying for proteins needed to reconstitute translocation-competent proteoliposomes upon detergent removal (*29*–*31*). In bacteria, three membrane-embedded proteins—SecY, SecE, and SecG—are tightly associated as a complex termed SecYEG (Fig. 2). Proteoliposomes bearing SecA:SecYEG will efficiently translocate pure preprotein, driven by ATP and a membrane potential (*29*). About 20 amino acyl residues are translocated for each ATP that is bound and hydrolyzed by SecA (*32*) in a cycle accompanied by substantial SecA conformational change (*33*). Each SecA translocates a preprotein through a single SecYEG (*34*, *35*), and the translocation pathway appears, by crystallography (*36*) and cross-linking (*37*), to pass through the center of SecY. The structure of SecYEG (*36*) shows a narrow constriction through which a polypeptide chain may move, rather than a large opening that would leak small molecules, and has provided the first molecular model of how apolar domains may be laterally released into the lipid bilayer. Cocrystals of SecYEG with SecA and with preprotein substrate may lead to further molecular understanding of the translocation cycle.

Fractionated detergent extracts of eukaryotic ER also yield a membrane-embedded heterotrimeric complex, termed Sec61αβγ, with marked similarity to SecYEG (*30*). Proteoliposomes bearing this Sec61 complex and the SRP receptor will translocate nascent chains initiated in the presence of SRP. The complex of polysome, nascent preprotein, and SRP binds to the SRP receptor. Upon the binding of guanosine 5′-triphosphate (GTP) to both the SRP and its receptor (*38*, *39*), the polysome and nascent chain are transferred to the Sec61 complex, allowing translation and trans-

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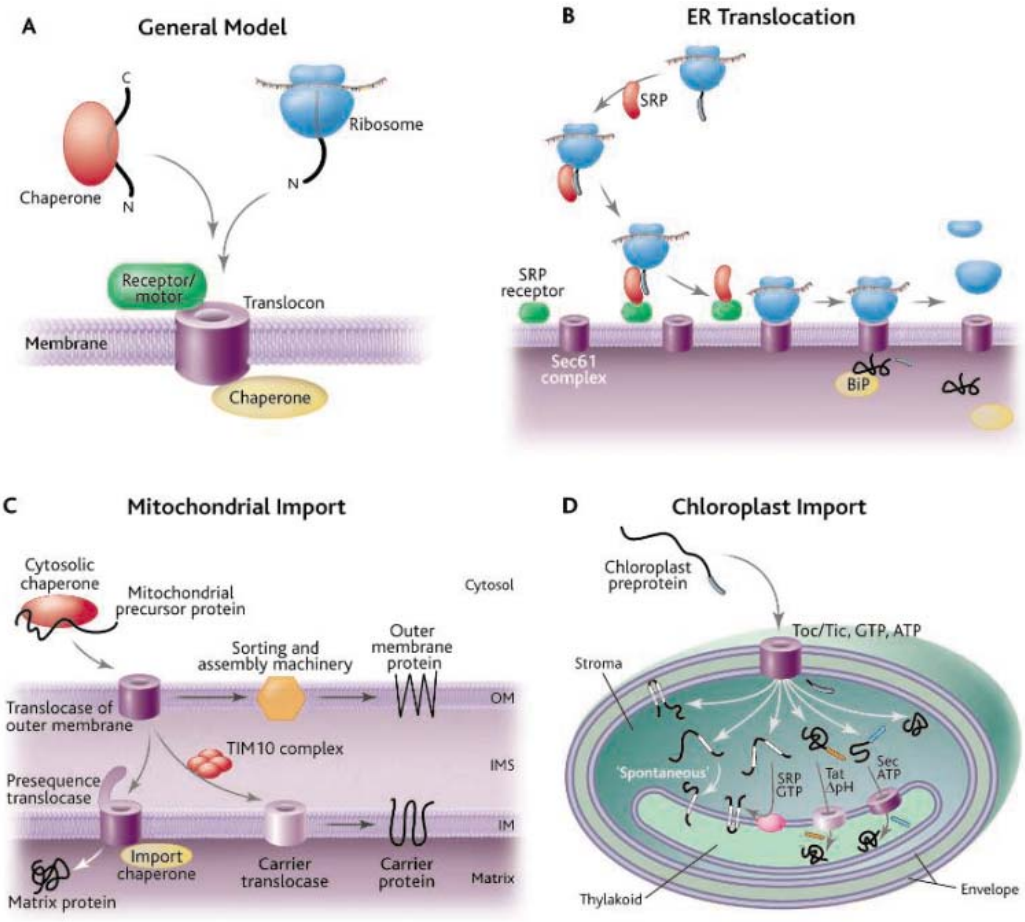

Fig. 1. Conserved translocation themes. (**A**) Either chaperones (red) or occupancy of the C-terminal region of nascent chains in the ribosomal exit tunnel (blue) prevents premature stable folding of preproteins. Membrane-bound receptors (green) bind preproteins and transfer them into the translocon (purple), which can conduct them across the bilayer or release apolar regions laterally into the bilayer. Motors and chaperones (yellow) on the trans surface of the membrane complete the transport task. (**B**) Cotranslational translocation into the ER. (**C**) Mitochondrial protein import [adapted from Wiedemann *et al.* (*94*)]. OM, outer membrane, IMS, intermembrane space, IM, inner membrane. (**D**) Chloroplast protein import [adapted from Jarvis and Robinson (*56*)].

location to resume (Fig. 1B). Preproteins translocate through the Sec61 complex or SecYEG translocons in the N-to-C direction. When ~20 largely apolar amino acyl residues enter the translocon, they are released sideways into the lipid bilayer.

It is possible that the chaperone systems for bacterial and eukaryotic ER translocation are not really so different. SRP-mediated translational arrest is not required for translocation (*40*, *41*); SRP and the ribosome (*42*) may be viewed as targeting chaperones. Yeast prepro-α factor and prepro-carboxypeptidase Y can translocate into the ER posttranslationally (*43*–*45*), and *Saccharomyces cerevisiae* can grow, albeit slowly, without SRP or its receptor (*46*, *47*). Bacterial SRP and SRP receptor are required for the assembly of many hydrophobic proteins into the membrane (Fig. 2) (*48*) but generally not for export to the periplasm or outer membrane (*49*). After import across the two envelope membranes, chloroplasts use SRP and its conserved receptor to assemble proteins into the thylakoid membrane (Fig. 1D) (*50*). This SRP-mediated thylakoid insertion is clearly posttranslational, emphasizing the receptor and chaperone roles of SRP. Targeting and chaperone functions are at the core of both cotranslational and posttranslational translocation pathways; irreversible folding or the aggregation of apolar membrane anchors can be prevented by engaging nascent polypeptides with the translocase as soon as its signal sequence emerges from the ribosome, whereas posttranslational translocation uses chaperones to target proteins and maintain translocation competence.

Import to the Mitochondrial Matrix

Mitochondrial protein uptake (Fig. 1C) is largely posttranslational, in vivo and in a reconstituted in vitro reaction (*13*, *14*, *51*, *52*). Mitochondria import 99% of their proteins posttranslationally from the cytosol, including integral membrane proteins of both the inner and outer membrane. Mitochondrial matrix preproteins have a distinct signal sequence, an amphipathic α-helical rod. Matrix preproteins bind to receptors on the surface of the mitochondrial outer membrane (*53*). They then enter a common translocation channel [the translocase of the outer membrane (called TOM)] to the intermembrane space. Preproteins translocate across the outer membrane in an unfolded state in an N-to-C direction. The membrane protonmotive force drives the initial stage of transport across the inner mitochondrial membrane through a distinct multisubunit complex, the translocase of inner membrane (TIM). As proteins enter the matrix, they are captured by mHsp70, a TIM-associated ATP-driven chaperone that binds each segment of the chain as it enters, thereby restricting net movement to import (*54*). During import, mitochondrial proteins may span both membranes with large C-terminal folded domains still exposed to the cytoplasm and their N terminus in the matrix; the matrix ATP-driven chaperones can then actually drive a net unfolding of the C-terminal domains (*55*).

The Diversity of Transport Systems

Mitochondria, gram-negative bacteria, and chloroplasts (*56*) have multiple, and branched, translocation pathways. Upon reaching the intermembrane space, mitochondrial preproteins enter divergent pathways (Fig. 1C): β-barrel proteins integrate into the outer membrane by means of a specialized outer membrane translocase (*57*, *58*); some proteins remain in the intermembrane space; and proteins with matrix-targeting presequences use the TIM translocase, exploiting two energy sources, the membrane potential $\Delta\Psi$ and the mHsp70 adenosine triphosphatase (ATPase). Apolar inner membrane proteins use a separate inner membrane translocase system that needs $\Delta\Psi$ but not

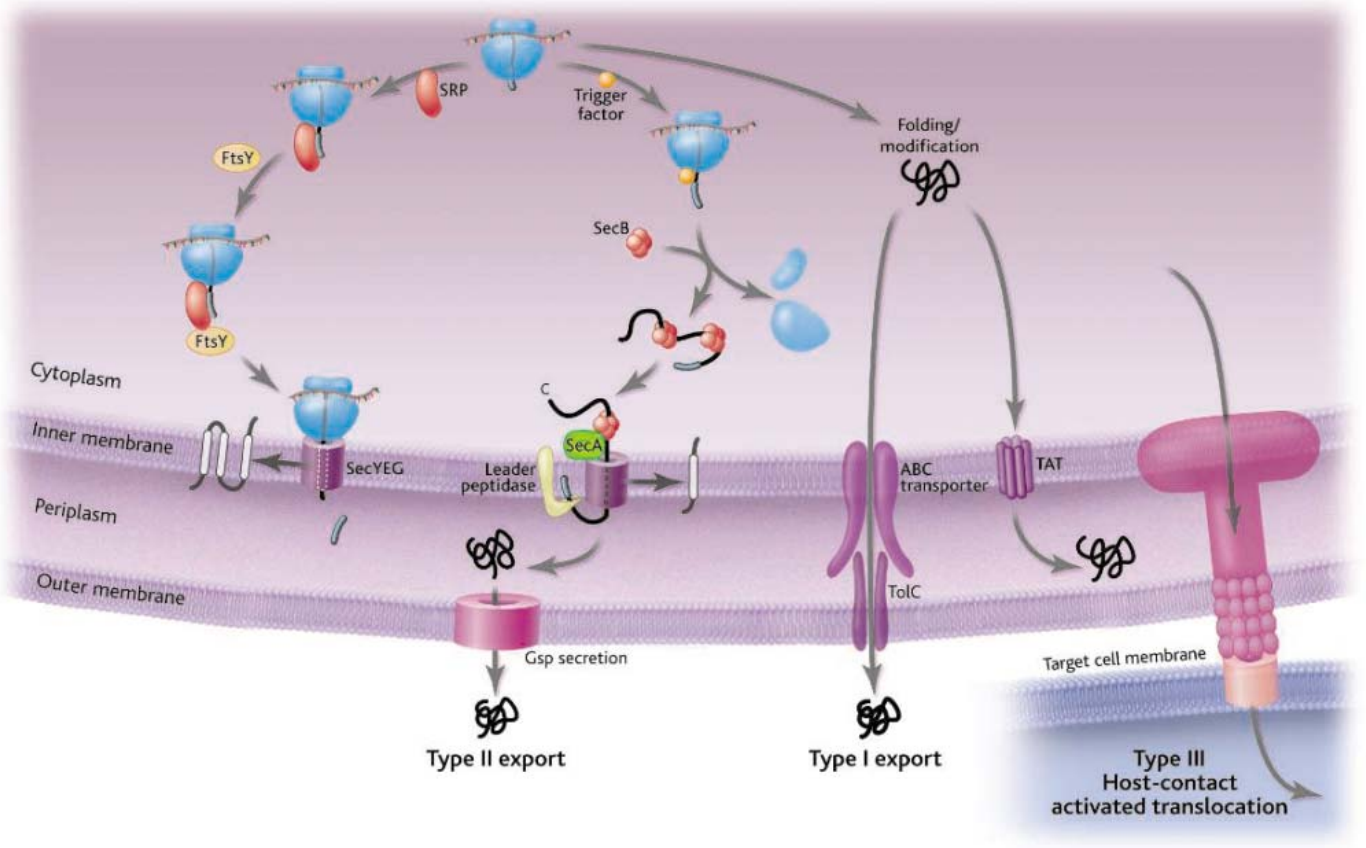

Fig. 2. Diversity of bacterial translocation pathways. [Adapted from Luirink and Sinning (*95*)]

ATP (*59*). Several particularly apolar proteins, encoded by the mitochondrial genome, are synthesized in the matrix and inserted into the inner membrane with the help of an inner membrane protein, Oxa1p (*60*). Other multimembrane systems are just as complex. Bacteria have the Sec translocase, TAT translocase, and YidC [homologous to Oxa1p (*61*)] for proteins entering the plasma membrane or periplasm, five transport systems for crossing the outer membrane, and distinct transport systems for coordinated transport across the inner membrane, outer membrane, and a target-cell membrane (Fig. 2). Chloroplasts (Fig. 1D) import proteins across two membranes and into the stroma by a coupled outer membrane and inner membrane transit pathway (*56*). From the stroma, import pathways into the thylakoid membrane and lumen are varied but markedly similar to bacterial protein export.

Not all protein translocation is dependent on an unfolded conformation. The TAT bacterial translocase exports proteins bearing a unique twin-arginine motif (*62*). Not only is this translocation uncoupled from ongoing protein synthesis, but it accommodates fully folded proteins that remain folded during membrane transit. The proteins translocated by TAT can be oligomeric, with some subunit(s) providing the TAT recognition motif, whereas others are translocated "piggyback," solely by virtue of their association with the TAT-motif–tagged subunit (*63*). TAT translocase subunits can oligomerize, suggesting a means for providing large transport pores (*64*). Oligomeric folded proteins are also imported into the peroxisome. Translocation is not limited to a single membrane or two membranes; chloroplasts have three distinct membrane layers, with unique aqueous spaces between each, and the type III transport systems of pathogenic bacteria can inject proteins across both bacterial envelope bilayers as well as across the target-cell plasma membrane.

Peroxisome Assembly

Peroxisomes display a curious patchwork of translocation mechanisms that resemble aspects of bacterial, mitochondrial, and nuclear translocation. Import of peroxisomal proteins is posttranslational and requires a short C-terminal signal sequence (-SKL is common) that is decoded by a receptor, the Pex5 protein (*65*, *66*). Most peroxisomal proteins are imported as an uncleaved mature species (*67*); certain proteins are even imported as oligomeric complexes (*68*). Soluble peroxisomal precursors may combine with a signal-specific receptor in the cytoplasm, enter the peroxisome with this receptor, and then release, allowing receptor recycling to the cytoplasm (*69*). Peroxisomal proteins, similar to nuclear proteins, fold in the cytoplasm and retain a native conformation during import. Though nuclear proteins traverse a large nuclear pore, no such morphological feature has been described for the peroxisome. Despite a rich list of genes (PEX) and proteins (peroxins) involved in peroxisome biogenesis, the translocation channel remains elusive. Several integral membrane proteins, including Pex3, Pex10, Pex15, and Pex19, are candidates (*70*). Although most peroxisomal matrix and membrane proteins are assembled into a mature organelle, the true origin of the peroxisomal membrane and at least one peroxin, Pex3, has remained controversial.

Until recently, the prevailing view has been that peroxisomes are self-renewing autonomous organelles. Blocks in secretion appear to have no effect on peroxisome proliferation (*71*). Many peroxisome assembly mutants produce ghostlike membrane remnants that may explain how PEX mutants restore normal peroxisome function on reintroduction of the missing peroxin (*70*). However, other results suggest a role for some other organelle as the origin of peroxisomal membrane. Peroxisomes in *S. lipolytica* contain glycoproteins, suggesting origins in the

early secretory pathway (*72*). *S. cerevisiae* pex3 null mutant cells have no apparent vestige of peroxisomal membrane, yet expression of Pex3 in this strain induces peroxisome proliferation (*73*).

This conundrum has apparently been resolved by the observation that Pex3 inserts into the ER membrane and then is diverted into vesicles that join by homotypic fusion or fuse with existing peroxisomes (*73*). Thus, Pex3 may exploit the secretory pathway to form new peroxisomes. The role of the ER Sec61 channel and of ER vesicle budding proteins in this process remains to be established. Activation of protein import in newly minted peroxisomes also remains an unexplored question.

Protein Dislocation

Eukaryotic cells use the cytoplasmic proteosome to degrade misfolded secretory glycoproteins, implying a retrograde translocation (or dislocation) of glycoproteins from the lumen of the ER to the cytosol (*74*–*76*). A number of proteins linked to this ER-associated degradation (ERAD) process have been identified in yeast and mammals (*77*). Dislocation requires luminal chaperone-like molecules that recognize and prepare misfolded proteins for export, integral membrane proteins that convey the misfolded proteins through the ER membrane, and cytoplasmic proteins that withdraw and covalently modify the substrates for degradation. Substrates include mutant and misfolded secretory and membrane proteins, bacterial toxins that penetrate to the cytoplasm and evade degradation, major histocompatibility complex (MHC) class I antigen that is diverted from the ER in cytomegalovirus-infected cells, metabolically regulated ER membrane enzymes such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and possibly even encapsulated virus particles that escape from the ER to replicate in the nucleus.

Although dislocation bears superficial similarity to translocation, the export pathway is not simply a reversal of import. Nevertheless, translocation and dislocation share some catalysts. Lumenal chaperones such as the hsp70-like BiP and protein disulfide isomerase recognize and may even initiate unfolding of dislocation substrates (*78*, *79*). Certain substrate molecules must acquire a specific glycan structure to be recognized for dislocation, and two glycan-binding proteins have been implicated in carbohydrate recognition (*80*–*82*).

Three distinct integral ER membrane proteins divert proteins for dislocation. One unique class, the cytomegalovirus gene products US2 and US11, binds to newly synthesized MHC class I receptor molecules in the ER (*83*, *84*). A complex between the class I protein and US11 associates with another ER protein called Derlin 1, initiating the diversion of class I protein from the ER. Derlin was first discovered as a yeast ER membrane protein, Der1. DER1 mutants block the ERAD of mutant secretory proteins but have no effect on the degradation of most membrane proteins targeted for destruction (*85*). There is also evidence that the ER protein import channel, Sec61, dislocates certain ERAD substrates (*86*,*87*). Indeed, there may be substantial differences in the mechanism of dislocation of membrane and soluble lumenal proteins, although we do not know enough to draw categorical distinctions. The actual channel for dislocation remains elusive.

Other proteins withdraw dislocating polypeptides from the ER membrane. First contact appears to be made by an AAA ATPase called p97 in mammals and Cdc48 in yeast. This complex may bridge Derlin 1 with the proteosome and a ubiquitination complex that covalently tags dislocating chains (*84*). Dislocation may be driven by ATP hydrolysis by p97, although not all substrates require the intervention of this chaperone. ERAD of unglycosylated α-factor precursor requires neither ubiquitin conjugation nor Cdc48. Indeed, ERAD of this substrate has been reconstituted with isolated ER membranes and the pure, intact proteosome complex (*87*). This reaction may be mediated by direct contact between the proteosome and the cytoplasmic face of the Sec61 channel (*88*).

Solutions to the Translocation Puzzles

How has nature answered each of the puzzling problems of translocation? Targeting is largely by recognition of small linear polypeptide domains, the ''signals.'' Many transport systems are designed only for unfolded polypeptide chains. For these translocons, preproteins either use chaperones to retain their capacity to be unfolded or couple their translocation to ongoing translation. Other translocases can carry even folded proteins across a membrane without lethal ion leakage, though TAT-dependent transport into thylakoids may leak as many as 30,000 protons (*89*). Translocons such as SecYEG or Sec61 complex can use either full-length proteins delivered by chaperones or nascent chains emerging from a polysome for which SRP and its receptor coordinate translation and translocation. This decision is probably made early in each protein's lifetime through a competition of ribosome-bound chaperones such as SRP or trigger factor for association with the emerging nascent chain (*90*). The energetics of transport are varied. ATP can power either SecA to ''push'' the translocation of ~20 amino acyl residues at a time or conserved Hsp70 ATPases which ''ratchet'' chains across the mitochondrial or ER membrane (*54*, *91*). The membrane electrochemical potential can directly act on transiting protein, ''electrophoresing'' its movement, and can promote the functional cycle of translocase proteins per se (*92*). Guanosine triphosphatases (GTPases) coordinate nascent preprotein delivery to the Sec61 complex (*38*) and regulate protein synthesis. Exploration of other membranes and organisms will likely reveal additional diverse translocation mechanisms.

Future Prospects

Structural biology may reveal how a motor such as SecA couples the movement of a preprotein segment to the energy of binding ATP or how SecYEG permits lateral exit of an apolar polypeptide segment into the bilayer (*93*). How do mitochondrial preproteins move so far into the organelle before the energy of the inner membrane potential can capture them for further steps of import, and are the import proteins of the two membranes aligned to facilitate import? How are whole, large folded proteins ''swallowed'' by the TAT or peroxisome transport systems? Finally, dislocation and translocation require recognition, delivery, and energy coupling that work from the opposite sides of the membrane. Our understanding of retrotranslocation is still at an early stage.

Note added in proof: For more on SecYEG structure, see K. Mitra *et al*., *Nature* **438**, 318 (2005).

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REVIEW

The Ins and Outs of DNA Transfer in Bacteria

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Transformation and conjugation permit the passage of DNA through the bacterial membranes and represent dominant modes for the transfer of genetic information between bacterial cells or between bacterial and eukaryotic cells. As such, they are responsible for the spread of fitness-enhancing traits, including antibiotic resistance. Both processes usually involve the recognition of double-stranded DNA, followed by the transfer of single strands. Elaborate molecular machines are responsible for negotiating the passage of macromolecular DNA through the layers of the cell surface. All or nearly all the machine components involved in transformation and conjugation have been identified, and here we present models for their roles in DNA transport.

In bacteria, transformation and conjugation usually mediate the transport of single-stranded DNA (ssDNA) across one or more membranes. Transformation involves the uptake of environmental DNA, whereas conjugation permits the direct transfer of DNA between cells (Fig. 1). Other DNA-transport phenomena in bacteria, such as the passage of DNA through the bacterial division septa and those carried out by many bacteriophages (*1*), involve the movement of double-stranded DNA (dsDNA) and will not be discussed here. Transformation and conjugation probably evolved for the acquisition of fitness-enhancing genetic information, but other mutually nonexclusive theories posit that transformation might have evolved to provide templates for DNA repair or to supply nutrition for bacteria (*2*). Today, both processes are recognized as important mechanisms for horizontal gene transfer and genome plasticity over evolutionary history, and they are largely responsible for the rapid spread of antibiotic resistance among pathogenic bacteria (*3*, *4*).

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Bacterial Transformation

Naturally transformable bacteria acquire a physiological state known as "competence" through the regulated expression of genes for protein components of the uptake machinery. Natural transformation has been most studied in *Bacillus subtilis*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae*. These and other competent bacteria use similar proteins for DNA uptake, with few differences between species. An interesting exception is *Helicobacter pylori*, which uses a conjugation-like system for transformation (*5*). Here, we will discuss the DNA uptake systems of *B. subtilis* and *N. gonorrhoeae* as representative of those in Gram-positive and -negative bacteria, respectively (Fig. 1A). The main distinction between these cell types is that Gram-negative bacteria are enclosed by cytoplas-

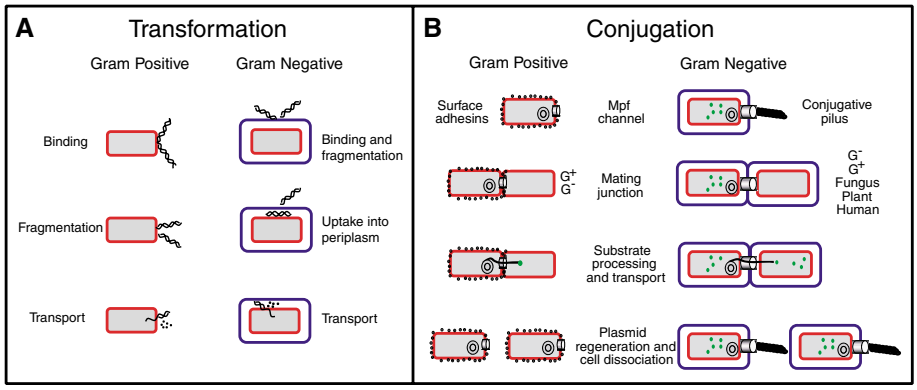

Fig. 1. Comparison of DNA processing and transfer during transformation and conjugation. (**A**) In transformation, dsDNA substrates are converted to single-stranded transfer intermediates for transport across the cytoplasmic membrane. (**B**) For conjugation, surface adhesins or conjugative pili mediate donor-target cell contacts. Initial reactions involve the formation of a relaxase–T-DNA transfer intermediate (green dot joined to black line) and tight mating junctions. Substrate transfer is probably mechanistically conserved in bacteria, although Gram-negative systems can deliver substrates, including proteins (green dots), to phylogenetically diverse target cells (*77*–*80*).

mic and outer membranes, with an intervening periplasmic space and thin layer of peptidoglycan (~3 to 7 nm) (*6*). Gram-positive bacteria lack an outer membrane, and their cytoplasmic membrane is surrounded by a ~22-nm periplasmic space and a thick layer of peptidoglycan (~33 nm) (*7*).

Initial interactions with the bacterial surface. In both Gram-positive and Gram-negative bacteria, dsDNA interacts with the competent cell surface by a process that is not completely understood. DNA binds to competent *B. subtilis* cells in a state that is resistant to centrifugal washing but susceptible to added nucleases. ComEA, a membrane-bound dsDNA binding protein, is required for transformation (*8*, *9*). In the absence of ComEA, 20% residual DNA binding still occurs in a competence-dependent manner (*8*). Similar results were observed in *S. pneumoniae* (*10*), but the proteins responsible for this residual binding remain unidentified in both species. In Gram-negative bacteria, dsDNA enters the periplasm, but in both Gram-negative and -positive systems, a single strand of DNA passes across the cytoplasmic membrane while its complement is degraded (Fig. 1A). DNA is taken up into the cytosolic space linearly (*11*), and a free end is presumably required to initiate the transport process. In *B. subtilis*, new termini are provided by random cleavage events on the cell surface, catalyzed by the integral membrane nuclease NucA (*12*).

Efficient DNA uptake in *Neisseria* and *H. influenzae* requires a species-specific DNA uptake sequence about 10 nucleotides long (*13*, *14*). The genomes of these bacteria are enriched for their respective uptake sequences, favoring the uptake of homospecific DNA (*15*). However, sequence-specific binding receptors have not yet been identified.

Secretins and uptake into the periplasm. In Gram-negative bacteria, dsDNA becomes nuclease-resistant as it passes through the outer membrane (Fig. 1A). This step requires the presence of a secretin protein (*16*). Secretins form stable, donut-like multimers in the outer membrane, with an aqueous central cavity (*17*). Secretins are also components of type-4 pilus, filamentous phage-extrusion systems, and dedicated protein-secretion systems, and they are also likely required for conjugation. For transformation, DNA probably enters the periplasm through the secretin channel, although direct evidence is lacking. The central cavity of the PilQ dodecamer is 6.5 nm in diameter at its widest point (*17*), adequate for the passage of dsDNA (2.4 nm) or of a DNA-protein complex.

The competence pseudopilus. Transformation systems of Gram-negative and -positive bacteria are made up of subunits with striking similarities to those needed for assembly of type-4 pili and type-2 secretion systems. Type-4 pili are long and thin appendages that mediate a form of locomotion known as twitching motility, which is powered by the extension and retraction of the pilus through assembly and disassembly. Type-2 secretion systems export folded-protein substrates across the outer membrane through a secretin channel. The conserved proteins for all three systems include a cytoplasmic adenosine triphosphatase (ATPase) of the AAA^+ ATPase superfamily (ATPases associated with various cellular activities), a polytopic membrane protein, a pre-pilin peptidase, and several pilins or pilin-like proteins (*18*). In type-4 pilus systems, these proteins mediate the assembly of the major pilin into the pilus fibers. Genetic manipulation, e.g., pilin overproduction, of a number of type-2 secretion systems also results in the production of pilus-like structures, termed pseudopili (Ψ-pili), that extend through the periplasm and in some cases beyond the cell surface (*19*–*22*).

In *B. subtilis*, the ComG proteins necessary for DNA binding (*23*) include the AAA^+ ATPase (ComGA), polytopic membrane protein (ComGB), major pre-pilin–like protein (ComGC), and three minor pre-pilin proteins (ComGD, ComGE, and ComGG) (Fig. 2). The pre-pilin proteins integrate into the cytoplasmic membrane, and when processed by the peptidase ComC, these subunits translocate to the exterior of the membrane (*24*). Recently, a polymeric complex dependent on the ComG proteins has been detected on the exterior of the membrane (*25*). This structure, termed a competence Ψ-pilus, consists of processed ComGC molecules joined to one another by disulfide bonds and by additional noncovalent interactions. The competence Ψ-pilus ranges in sizes corresponding to 40 to 100 subunits and, on the basis of length estimates for a secretion Ψ-pilus (*22*) and type IV pili (*26*), the competence Ψ-pilus is long enough to traverse the periplasm and cell wall (~55 nm) (*7*).

N. gonorrhoeae produces type-4 pili, and many proteins needed for pilus formation are also required for DNA uptake and transformation, leading to the assumption that pili participate in DNA uptake. However, there is evidence that two distinct structures exist in *Neisseria*, the type-4 pilus and a competence Ψ-pilus, and that these structures apparently compete for common components and morphogenetic proteins (*27*, *28*).

The growing secretion Ψ-pilus may act as a piston, pushing substrate proteins through the secretin channel in the outer membrane (*18*, *29*, *30*). Analogously, assembly and disassembly of the competence Ψ-pilus may contribute to DNA uptake by pulling DNA to the translocation machine in the cytoplasmic membrane (Fig. 2). Repeated cycles of assembly and disassembly would result in a low concentration of maximal-length Ψ-pilus and a broad size distribution, as observed for the *B. subtilis* competence Ψ-pili and the secretion Ψ-pili of *Xanthomonas campestris* (*21*). In single-molecule studies of DNA uptake in *B. subtilis* (*31*), the rate of uptake (~80 base pairs s^{-1}) was relatively constant with forces up to 40 pN, without detectable pauses or reversals. These features, unusual for molecular motors that move along DNA, are similar to the force characteristics of type-4 pilus retraction in *N. gonorrhoeae* (*32*). The proton motive force may be a source of energy for DNA uptake; the rate of uptake decreases sharply with the addition of uncoupling agents before any detectable decline in the ATP pool (*31*). Thus, the proton motive force might directly drive the movement of the Ψ-pilus subunits into the membrane, causing Ψ-pilus disassembly and retraction.

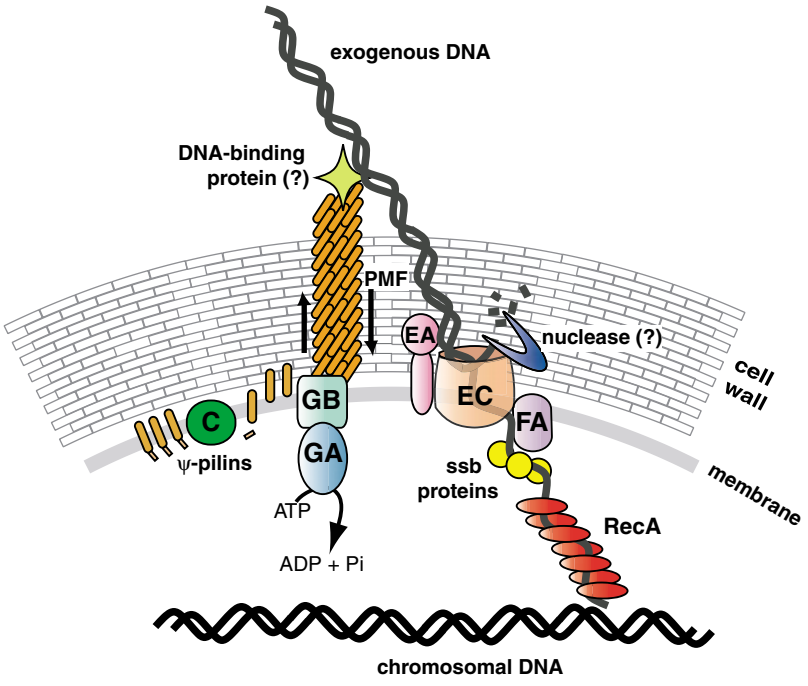

Fig. 2. DNA uptake during transformation in *B. subtilis*. The uptake machinery is preferentially located at the cell poles. The Ψ-prepilins are processed by the peptidase and translocate to the outer face of the membrane. With the aid of the other ComG proteins, the major Ψ-pilin ComGC assembles into the Ψ-pilus, which attaches exogenous DNA via a hypothetical DNA binding protein. Retraction of the Ψ-pilus, driven by the proton motive force, and DNA binding to the receptor (ComEA) are required to transport one strand of DNA through the membrane channel (ComEC) while the other is degraded by an unidentified nuclease. The helicase/DNA translocase (ComFA) assists the process, along with ssDNA binding proteins that interact with the incoming DNA. RecA forms a filament around the ssDNA, and mediates a search for homology with chromosomal DNA. ADP, adenosine diphosphate; Pi, inorganic phosphate; PMF, proton motive force; ssb, single-stranded DNA binding protein.

Transport across the cytoplasmic membrane and DNA processing. In both *B. subtilis* and *N. gonorrhoeae*, similar polytopic membrane proteins (ComEC and ComA, respectively) are required for DNA transport into the cytosol (*8*, *33*). These large proteins (ComEC contains 776 residues) are proposed to form channels for the passage of DNA (*34*). In addition, the Gram-positive systems encode a membrane-bound ATPase, ComFA, that functions in DNA uptake (*35*). ComFA resembles the family of Asp-Glu-Ala-Asp (DEAD) box helicases, and may assist the translocation of DNA through the membrane or carry out strand separation. In *S. pneumoniae*, the membrane-associated EndA nuclease degrades the nontransforming strand, even when the ComEC equivalent is absent (*10*). In *B. subtilis*, the identity of the corresponding nuclease is unknown, but degradation of the nontransforming strand seems dependent on passage through or interaction with ComEC (*12*).

Cellular location of DNA uptake and the role of cytosolic proteins. In *B. subtilis*, which is a rod-shaped bacterium, DNA binding and uptake take place preferentially at the cell poles, where the membrane-associated proteins ComGA and ComFA and the cytosolic ssDNA binding protein YwpH colocalize (*36*). Several additional cytosolic proteins participate in transformation, and some have been shown to associate with ssDNA entering the cell. In *S. pneumoniae*, the Smf protein protects transforming DNA from degradation in the cytosol (*37*). The repair/recombination proteins RecN and RecA also localize to the poles of competent *B. subtilis* (*38*). RecN oscillates from pole to pole, but becomes static at one pole when transforming DNA is added. RecA localization depends on ComGA; when DNA is added, RecA forms a filament extending from the pole to the centrally located nuclear body, perhaps facilitating the search for a homologous site on the chromosome.

A transformation model. We propose that repeated cycles of Ψ-pilus assembly and disassembly drive a DNA molecule through the cytoplasmic membrane channel formed by ComEC and that an unidentified DNA binding protein anchors DNA to the Ψ-pilus. ComEA may ensure processivity by maintaining contact with DNA as these cycles push DNA through the channel (*8*). The proton motive force might drive disassembly of the Ψ-pilus. Finally, the binding energy of cytosolic ssDNA binding proteins might provide a pulling force by a Brownian ratchet mechanism (*39*), and the helicase/translocase ortholog ComFA may also assist uptake.

Conjugation

Most bacterial and some archaeal species encode conjugation systems, and several classes of mobile elements exist, including self-transmissible and mobilizable plasmids, conjugative transposons, and integrative conjugative elements (*40*). We will restrict the discussion to a few of the better-characterized, plasmid-encoded conjugation systems of Gram-negative bacteria and draw on examples from the Gram-positive bacteria where information is available.

The conjugation apparatus is composed of a cell-envelope–spanning translocation channel and either a pilus for Gram-negative bacteria or surface-localized protein adhesins for Gram-positive bacteria (Fig. 1B) (*41*, *42*). The mating pair formation (Mpf) proteins elaborate the extracellular pilus, and these subunits plus the coupling protein, here termed the substrate receptor, mediate substrate transfer across the cell envelope (*43*, *44*). *Agrobacterium tumefaciens* elaborates a model conjugation machine from 11 Mpf subunits, VirB1 to VirB11, and the VirD4 substrate receptor, to deliver oncogenic transferred DNA (T-DNA) to susceptible plant species (*45*). Many plasmid conjugation systems, exemplified by transfer systems of plasmids R388, F, and RP4, are built from nearly complete sets of VirB/D4-like subunits, whereas other systems have only one or two discernible homologs (*41*, *42*, *44*–*47*). Conjugation and ancestrally-related translocation machines make up the large and functionally versatile family of type-IV secretion systems (*46*–*48*).

Processing of the conjugative-transfer intermediate. The processing of substrate DNA for conjugative transfer is a widely conserved reaction among Gram-negative bacteria and unicellular Gram-positive bacteria (Fig. 1B) (*49*). A relaxase plus one or more auxiliary factors initiate processing by binding the origin-of-transfer (*oriT*) sequence and cleaving the DNA strand destined for transfer (T-strand). The relaxase remains covalently bound to the 5′ end of the T-strand, resulting in the formation of the relaxase–T-strand transfer intermediate. This processing reaction clearly is distinct from the strand-specific degradation pathways operating during transformation. Also in contrast to the competence systems, signals conferring substrate recognition are carried not by the DNA but by the relaxase; these minimally consist of positively charged or hydrophobic clusters of C-terminal residues and are found in other protein substrates as well (*50*–*52*). Conjugation systems thus are currently viewed as protein-trafficking sys-

tems that have evolved the capacity to recognize and translocate relaxases and, only coincidentally, "hitchhiker DNA" (*53*).

Definition of a DNA substrate translocation route. The processed DNA substrates are recruited to the cognate conjugation apparatus by VirD4-like receptors. These receptors are multimeric ATPases (*54*), and they are defining components of Gram-negative and -positive conjugation systems (*43*, *55*–*59*). Members of this protein family might also function as cytoplasmic membrane translocases, which is suggested by a structure of the TrwB receptor of plasmid R388 presenting as a spherical homohexamer with an N-terminal transmembrane stem and a central 2-nm channel (*43*, *59*). However, VirD4 receptors cannot mediate transport independently of the Mpf proteins, e.g., VirB components. In *A. tumefaciens*, the VirD2 relaxase–T-strand intermediate forms a series of spatially and temporally ordered close contacts with six VirB/D4 machine subunits during translocation (Fig. 3) (*58*). Upon substrate docking, VirD4 delivers the transfer intermediate to the VirB11 ATPase, a member of the AAA^+ superfamily positioned at the inner face of the cytoplasmic membrane (*60*, *61*). This reaction proceeds in the absence of ATP use by VirD4 and VirB11, but requires several other subunits distributed across the cell envelope that probably contribute to the structural integrity of the cytoplasmic membrane translocase (*62*, *63*).

Next, the relaxase–T-strand is delivered sequentially to the integral cytoplasmic membrane components VirB6 and VirB8 by mechanisms dependent on ATP energy consumption by VirD4, VirB11, and a third ATPase of this system, VirB4 (Fig. 3) (*62*). VirB6 is a polytopic membrane protein and might function as a water-filled channel through which the substrate passes, reminiscent of *B. subtilis* ComEC discussed above (*64*). Finally, the substrate is delivered to two periplasmic/outer membrane–bound subunits, VirB2 pilin and VirB9. On the basis of the demonstrated substrate contacts, VirD4, VirB11, VirB6, VirB8, VirB2, and VirB9 are postulated to make up the mating channel for DNA transfer across the *A. tumefaciens* cell envelope (*58*). Gram-positive systems possess VirD4- and VirB4-like subunits that probably also form part of the membrane translocase (*42*).

Structural and energetic requirements for substrate translocation through the periplasm and outer membrane. The mating channel extending through the periplasm has been depicted as a rudimentary pilus (Fig. 3) (*40*, *44*, *45*). Alternative models exist, most notably one postulating that the VirB2 pilin undergoes cycles of assembly and disassembly to form a dynamic piston (*47*). This model is reminiscent of that proposed above for the competence Ψ-pilus, but here a VirB2 piston would supply the force needed for passage of DNA across the outer rather than the cytoplasmic membrane. VirB9-like subunits presently are the best candidates among the VirB components for forming an outer-membrane pore or channel (*41*, *45*). These outer-membrane components share sequence similarities with the pore-forming secretins and, like secretins, they often form stabilizing interactions with cognate lipoproteins (*41*, *45*, *65*). *A. tumefaciens* VirB9 confers selective trafficking of different relaxase–T-strand substrates through the distal portion of the secretion channel, also reminiscent of substrate-specifying activities reported for secretins (*66*, *67*). VirB2 pilin and VirB9 secretin-like components are found in nearly all conjugation systems of Gram-negative bacteria but not Gram-positive bacteria, suggesting that mechanistic differences probably exist for translocation to the surfaces of these cell types (*42*, *45*).

Both ATP energy and proton motive force are needed for conjugative DNA transfer (*68*). In *A. tumefaciens*, the VirD4 and VirB11 ATPases convert ATP energy to a mechanical force by inducing a structural transition in the cytoplasmic membrane subunit VirB10 (Fig. 3) (*69*). In turn, energized VirB10 forms a stable complex with secretin-like VirB9 at the outer membrane. VirB9-VirB10 complex formation is a prerequisite for the passage of DNA substrates from the portion of the channel composed of VirB6 and VirB8 at the cytoplasmic membrane to that composed of VirB2 and VirB9 (*69*). Energized VirB10 might physically bridge machine subassemblies at the two membranes or, alternatively, trigger gate opening at the distal portion of the secretion channel. Intriguing structural and functional similarities exist between VirB10-like subunits of conjugation systems and the TonB family of energy transducers, although the former sense ATP energy and the latter sense the proton motive force (*69*).

Roles of extracellular structures and the nature of the donor-recipient cell contact. In

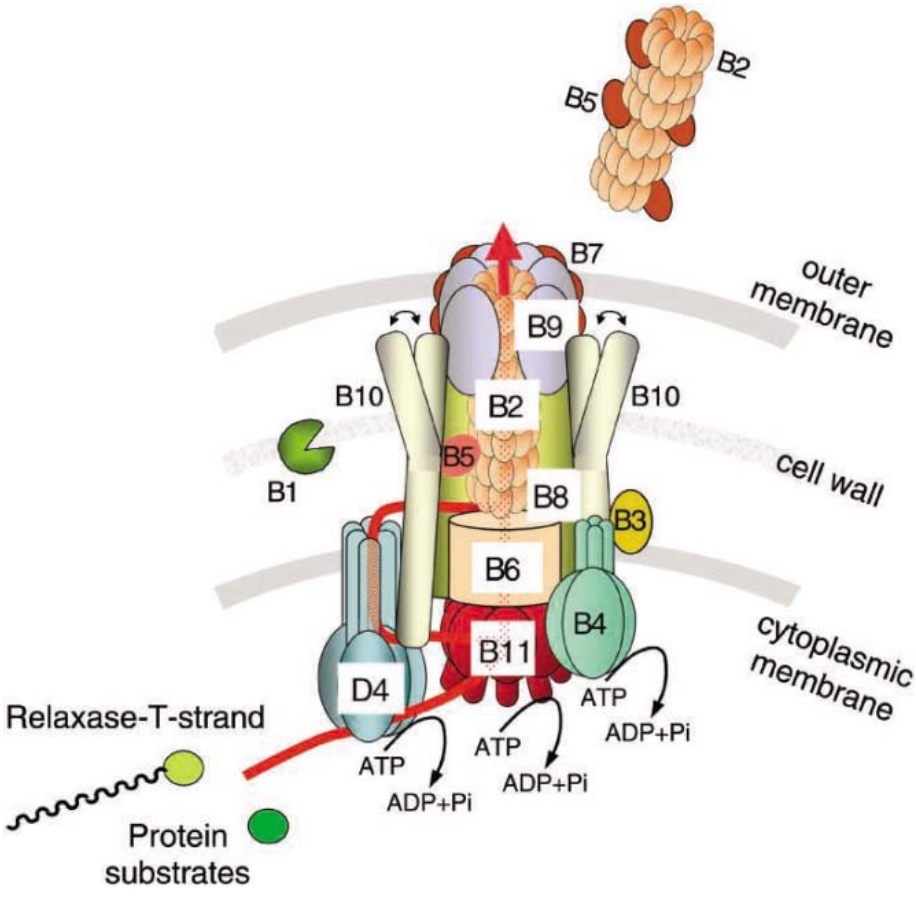

Fig. 3. Conjugative DNA transfer through the *A. tumefaciens* VirB/D4 system. DNA and protein substrates dock initially at the VirD4 receptor, then transfer in succession to the channel components VirB11 ATPase, VirB6, and VirB8, and finally VirB2 and VirB9. Three ATPases (VirD4, VirB4, VirB11) energize DNA substrate transfer through the membrane translocase comprised of either or both VirD4 and VirB6. The DNA substrate translocates to the cell surface via a channel comprised of VirB2 pilin and secretin-like VirB9. ATP energy also induces a structural transition (double-ended arrow) in VirB10 to mediate substrate transfer to the distal portion of the secretion channel.

Gram-negative bacteria, the pilus mediates initial attachment of donor cells with recipient cells. In the *Eschericia coli* F plasmid system, the pilus retracts and is postulated to function dynamically to bring donor and recipient cells into contact to form the mating junction (*41*). In contrast, the *A. tumefaciens* VirB/D4 system and related plasmid transfer systems, e.g., RP4, R388, and pKM101, lack the Mpf subunits dedicated to F pilus retraction (*70*), and these systems most probably release their pili from the cell surface either by breakage or an active sloughing mechanism (Fig. 3) (*44*, *45*). Such pili probably function as adhesive structures, resembling the surface adhesins of Gram-positive conjugation systems, e.g., *E. faecalis* pCF10-encoded aggregation substance, by promoting aggregation of donor and recipient cells (*71*).

Conjugative pili extending from the cell surface induce the formation of mating pairs but probably play no direct role in substrate transfer. Conjugative junctions visualized by electron microscopy appear as tightly apposed outer membranes devoid of structures, e.g., pili, and they typically exceed 100 nm along the cell length (*72*). These findings, plus new evidence for interactions between membrane proteins of donor and recipient cells (*73*), suggest that donor and recipient cell membranes might undergo extensive remodeling during the formation of mating junctions. Additionally, mutations in certain Mpf subunits of the plasmid RP4 and *A. tumefaciens* VirB/D4 machines genetically ''uncouple'' two pathways, one leading to the formation of the pilus, the other to a functional secretion channel (*45*, *74*). Thus, reminiscent of the neisserial competence and type-4 pilus systems discussed above (*28*), the Mpf subunits might assemble alternatively as a secretion channel or an extracellular pilus (*45*).

Spatial positioning of the conjugative transfer apparatus. Conjugation components and pili display both distributed and polar patterns of localization. Conjugation components and pili of the plasmid R27 system localize at many sites around the cell surface (*75*). In contrast, VirB subunits and pili of the *A. tumefaciens* VirB/D4 assemble at the cell poles (*76*). The VirD4 T4CP also is polar-localized where it recruits a green fluorescent protein (GFP)–tagged protein substrate, strongly suggesting that this is the site for translocation (*57*). In this plant pathogen, a polar-localized conjugation machine might have evolved as a specialized adaptation for substrate transfer to susceptible hosts.

Summary

The early reactions mediating processing of dsDNA to translocation-competent ssDNA substrates clearly are strikingly different for transformation and conjugation systems. Yet for both systems, the actual process of ssDNA transport across bacterial membranes might be more mechanistically conserved than previously envisioned. Both systems probably use similar strategies for substrate passage through the following: (i) the outer membranes of Gram-negative bacteria (via secretin complexes), (ii) the periplasm or cell wall (pilus- or Ψ-pilus–mediated), and (iii) the cytoplasmic membrane (at least in part through a water-filled channel composed of a polytopic membrane protein). Both systems also appear to use AAA^+ ATPases and proton motive force to induce dynamic structural changes for translocation. Finally, at least one conjugation-like machine, the *H. pylori* Com system, has evolved for DNA acquisition (*5*).

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Principles of Selective Ion Transport in Channels and Pumps

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The transport of ions across the membranes of cells and organelles is a prerequisite for many of life's processes. Transport often involves very precise selectivity for specific ions. Recently, atomic-resolution structures have been determined for channels or pumps that are selective for sodium, potassium, calcium, and chloride: four of the most abundant ions in biology. From these structures we can begin to understand the principles of selective ion transport in terms of the architecture and detailed chemistry of the ion conduction pathways.

The flow of ions across the cell membrane is essential to many of life's processes. Ion pumps build gradients across the membrane, which are then used as an energy source by ion channels and other transport proteins to pump nutrients into cells, generate electrical signals, regulate cell volume, and secrete electrolytes across epithelial layers (*1*). Life depends on the continued flow of ions into and out of cells. But the cell membrane presents a serious energy barrier to an ion crossing it (Fig. 1). This is because ions are energetically more stable in water than in the oily substance of the membrane interior: Outside the membrane, polar water molecules point their charged edges toward an oppositely charged ion, but inside the membrane such stabilizing interactions are reduced. The resulting energy difference is so large that the predominant ions in biological systems—Na^+, K^+, Ca^{2+}, and Cl^-—would essentially never cross the membrane unaided. Ion pumps, ion exchangers, and ion channels (membrane proteins that we refer to here as the ion-transport proteins) are used by the cell to transport ions across membranes. For simplicity, we group the "active" transport proteins—the ion pumps and ion exchangers—together into a single category called pumps.

Ion pumps and ion channels fulfill very different functions. The pumps transport ions against their electrochemical gradient by coupling the "uphill" transport process to an energy source such as adenosine triphosphate (ATP) hydrolysis or the "downhill" movement of another ion or substrate molecule. Ion channels by contrast are passive, simply catalyzing the downhill movement of ions, in many cases at very high ion conduction rates. Ion pumps and ion channels share one fundamental property: an ability to transport ions in a selective manner. Ion selectivity is crucial to the operation of ion-transport proteins.

Recent x-ray crystallographic studies have revealed at least one example of a transport protein for each of the predominant ions in biology. With the variety of architectural motifs now described, we can begin to see and appreciate that nature has come up with many different solutions for overcoming the energy barrier to allow an ion to cross the membrane. Here we will describe a subset of those solutions, with particular emphasis on the chemical principles by which ions are transported selectively across the membrane.

Architecture

To understand how ion channels and ion pumps catalyze the passage of substrates across the membrane bilayer, we can begin by studying the proteins' architectures and shapes and how their shapes are related to the passageway across the membrane bilayer. Drawing from the potassium and chloride channel (ClC) families, and together with the sodium-dependent glutamate transport homolog (Glt_{Ph}) and the Ca^{2+} ATPase, we see that in some cases the protein creates a proteinacious passageway spanning approximately the thickness of the entire membrane bilayer, whereas in other cases the protein creates aqueous cavities or vestibules, some of which reach more than halfway across the bilayer (Fig. 2), that are accessible from bulk solution. In these latter instances, ions and substrates reach selectivity filters or binding sites deep within the membrane by simple diffusion through the aqueous solution.

Perhaps one of the most striking examples of how the architecture of a pump partially solves the problem of moving ions and charged substrates across the membrane can be found in the case of Glt_{Ph}, a prokaryotic homolog of sodium-coupled glutamate transporters (*2*): proteins that couple the uphill movement of glutamate to the energetically favorable movement of sodium. Here, the homotrimeric protein creates an outwardly facing aqueous basin, nearly 50 Å in diameter, the bottom of which is located approximately halfway across the membrane bilayer (Fig. 2). Because the binding sites for the substrate are located at the bottom of the basin and are accessible from the external aqueous solution, the substrate can simply diffuse through bulk solution to a binding site halfway across the membrane. The voluminous basin of these pumps effectively displaces the outer leaflet of the membrane with aqueous solution so that the protein only need catalyze the passage of the substrate and ions across one leaflet of the bilayer.

Potassium ion channel proteins, exemplified by the prokaryotic channel KcsA (*3*), provide a second example of how fundamental elements of architecture are intimately related to mechanisms of transmembrane transport. KcsA has a wide water-filled pore facing the cytoplasm, which traverses more than half of the membrane bilayer, ending at the selectivity filter, near the negative end charges of the four pore helices (Fig. 2). This aqueous region of the pore allows ions to rapidly diffuse across about two-thirds of the membrane bilayer be-

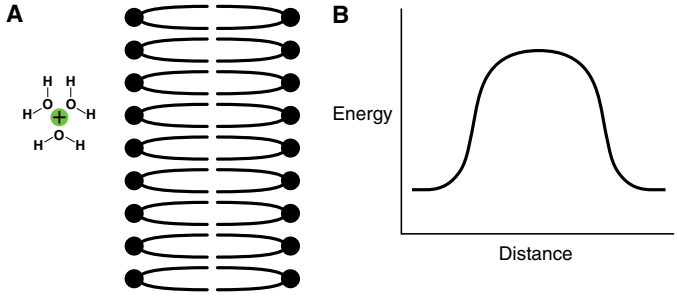

Fig. 1. The membrane presents an energy barrier to ion crossing. (**A**) Schematic of a positively charged cation (+ symbol) solvated by polar water molecules and of a membrane bilayer. (**B**) Simple graph showing that movement of the cation through the hydrophobic portion of the membrane bilayer is an energetically unfavorable process.

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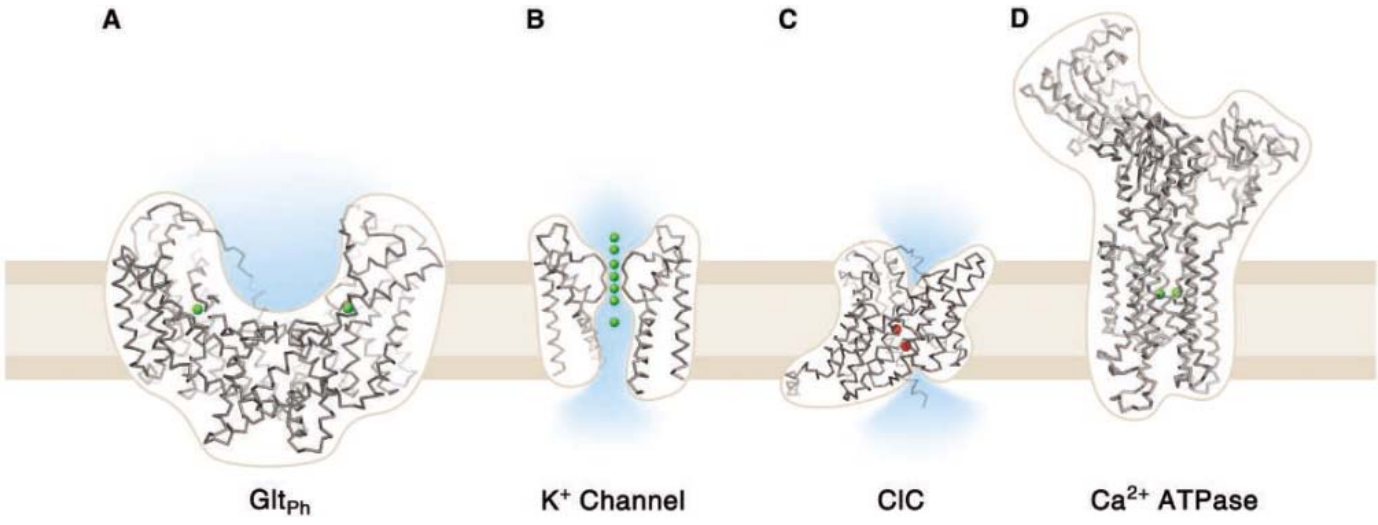

Fig. 2. Architectures of ion channel and ion pump proteins showing approximate locations of solvent-accessible or solvent-filled regions of the proteins (blue shading) and the relative position of the membrane (horizontal beige bands). (**A**) The bottom of the aqueous basin of the Glt_{Ph} pump lies near the middle of the bilayer and allows direct access, from bulk solution, to substrate (green spheres) and ion binding sites [Protein Data Bank (PDB) code 1XFH]. (**B**) An open-channel model of the KcsA K^+ channel, based on the structure of the MthK channel (PDB code 1LNQ), possesses a water-filled pore in the middle of the transmembrane portion of the channel and a selectivity filter near the outside occupied by K^+ ions (green spheres) (PDB code 1K4C). (**C**) Funnel-shaped structures are present in the ClC Cl transport proteins and allow ions (red spheres) to reach the selectivity filter, located at the midpoint of the bilayer (PDB code 1OTS). (**D**) A structure of the calcium-bound form of the Ca^{2+} ATPase shows that this transport protein does not have large solvent-filled cavities (PDB code 1SU4). Rather, the two bound ions (green spheres) occupy solvent-occluded sites within the transmembrane domains of the pump. In (A) to (C), the intracellular surfaces are "down," whereas in (D) the intracellular surface is "up."

fore reaching the selectivity filter. The selectivity filter, only ~12 Å in length, is the locus of ion discrimination and forms the remainder of the passageway across the membrane bilayer. In potassium channels, a long aqueous passageway connecting to a short selectivity filter is probably a key element of the channel architecture that contributes to the remarkably high flux rates of $\sim 10^8$ ions per second.

Not all ion-transport proteins have large aqueous vestibules within the transmembrane-spanning portion of the protein, however. The ClC chloride channels and chloride proton exchangers (ClC Cl transport proteins), in contrast to potassium channels, harbor double hourglass-like funnels at the extracellular and cytoplasmic surfaces, leading to a narrow constriction—the anion selectivity filter—located at the middle of the bilayer (*4*, *5*). When the channel is viewed parallel to the membrane (Fig. 2), one can see that the two funnels are related by a pseudo twofold axis, and indeed the protein folds of the amino and carboxy terminal halves of each subunit are related by a twofold axis. A roughly similar shape is also seen in the glycerol (*6*) and water channels (*7*, *8*), which suggests that double-funnel architecture may be a relatively common shape in channels and pumps. If this is the case, what purposes(s) might such architecture serve? On the one hand, the double-funnel architecture, together with the twofold axis parallel to the membrane, may facilitate the formation of ion binding sites and selectivity filters at the midpoint of the membrane bilayer that are at least partially composed of reentrant helices and loops. On the other hand, this architecture may also be rooted in evolution; perhaps the primordial ancestors of ClC proteins, as just one example, were about half the size of their modern-day counterparts and were bona fide dimers. Over time, gene fusion and mutation gave rise to the present-day proteins.

Knowing the structure of an ion channel or ion pump does not always illuminate how the ion or substrate reaches binding sites within transmembrane domains, and it appears that at least some pumps (the Ca^{2+} ATPase being an example) certainly do not possess tell-tale vestibules or channels and may have particularly cryptic pathways for substrate entrance and egress. Manifold studies of the Ca^{2+} ATPase, a paradigm of so-called P-type ATPases, still have not clearly revealed the route(s) by which calcium binds to and leaves from occluded transmembrane binding sites, even though there are crystal structures of nearly every intermediate along the transport pathway (*9*). All we know at present is that calcium may reach the intramembrane binding sites, from the cytoplasm, via a narrow pathway lined with acidic residues. Surprisingly, studies have not yet uncovered a convincing pathway from the occluded binding sites to the lumen, and thus conformational states not yet observed must exist to allow calcium to reach the lumen.

In spite of the remarkably variable architectures and shapes of channel and pump proteins, they can teach us some useful lessons. First, ion-channel proteins, whose business is typically to catalyze the selective passage of ions at a high rate, have wide regions of their pores that allow ions to remain solvated by water as long as possible before reaching constrictions that confer selectivity; these regions are elements of protein structure that may span only a fraction of the bilayer thickness. Second, although some pumps such as Glt_{Ph} have large basins that reach to substrate binding sites deep within the membrane, other pumps, such as the Ca^{2+} ATPase, do not. Furthermore, for Glt_{Ph} and the Ca^{2+} ATPase, there are no apparent pathways for ions and substrate to pass from occluded binding sites to the cytoplasm and lumen, respectively, and thus additional, as yet uncharacterized, conformational states must exist. Although the flux rates of channels and pumps vary by as much as 10^6-fold and they have completely different structures, both classes of proteins exhibit selectivity for their cognate substrate, and in the next section we examine the physical and chemical principles underlying ion selectivity in channels and pumps.

Ion Selectivity

Most transport proteins need to get certain ions across the membrane while at the same time excluding others. Such ion selectivity can be extremely precise, for instance, between ions as similar as Na^+ and K^+ (*1*). Ion selectivity between such similar ions requires the ion pathway to have specific binding sites over at least part of its length. These sites allow a transport protein to "feel" ions to ensure that only the right ones can pass. Of course, for an ion to be felt, it has to be dehydrated (at least partially if not completely),

and dehydration costs energy. Binding sites therefore have to compensate for the energetic cost of dehydration by providing favorable compensatory interactions with the ion. Selectivity results when this energetic compensation is more favorable for one type of ion than for another, relative to the energy of dehydration. Studies over the past 50 years on synthetic and naturally occurring ion-binding small molecules (host/guest chemistry with ions) have established the basic rules of ion selectivity within small molecules (*10*). Two major factors contribute to ion binding site selectivity: the atomic composition and the stereochemistry (e.g., size) of the binding site. Recently, protein structures have begun to show us for the first time how ion selectivity for Na^+, K^+, Ca^{2+}, and Cl^- ions is accomplished in membrane transport proteins.

Sodium and Potassium

The alkali metal ions Na^+ and K^+ are the most abundant cations in biological systems. Na^+ ions are most often present at high concentrations outside the cell, and K^+ is present at high concentrations inside. Gradients for these ions across the cell membrane provide the energy source for action potentials generated by opening Na^+ and K^+ channels, for synthesizing ATP in some organisms (*11*, *12*), and for moving solutes and other ions across the cell membrane via coupled transporters. The Na^+-selective binding sites in the Na^+-dependent leucine transporter LeuT (*13*) and the K^+-selective binding sites in the K^+ channel (*14*) provide a direct comparison of selectivity among these ions in transport proteins at high resolution.

LeuT transports leucine and Na^+ in the same direction across the cell membrane (*13*). By coupling the transport of leucine to Na^+, LeuT uses the energy of the Na^+ gradient to pump leucine into the cell. The atomic structure shows a leucine and two Na^+ ions bound deep inside the protein, partway across the membrane (Fig. 3A). The Na^+ ions are completely dehydrated. One site contains six oxygen atoms in direct contact with the ion. Five of these oxygen atoms bear only a partial negative charge (the main-chain carbonyl, side-chain hydroxyl, and side-chain amide atoms) and one bears a full negative charge on a carboxylate group. The second site contains five oxygen atoms surrounding the Na^+ ion. In this case, all the oxygen atoms bear only a partial negative charge (the main-chain carbonyl and side-chain hydroxyl atoms). Two important features of these binding sites are evident. First, the binding sites consist of oxygen atoms in direct contact with Na^+; a formal negative charge can occur (site 1) but is not essential (site 2). Second, the size of the binding site cavity formed by the oxygen atoms is a good match to the Na^+ ion, with a mean Na^+-O distance for both sites combined of 2.28 Å.

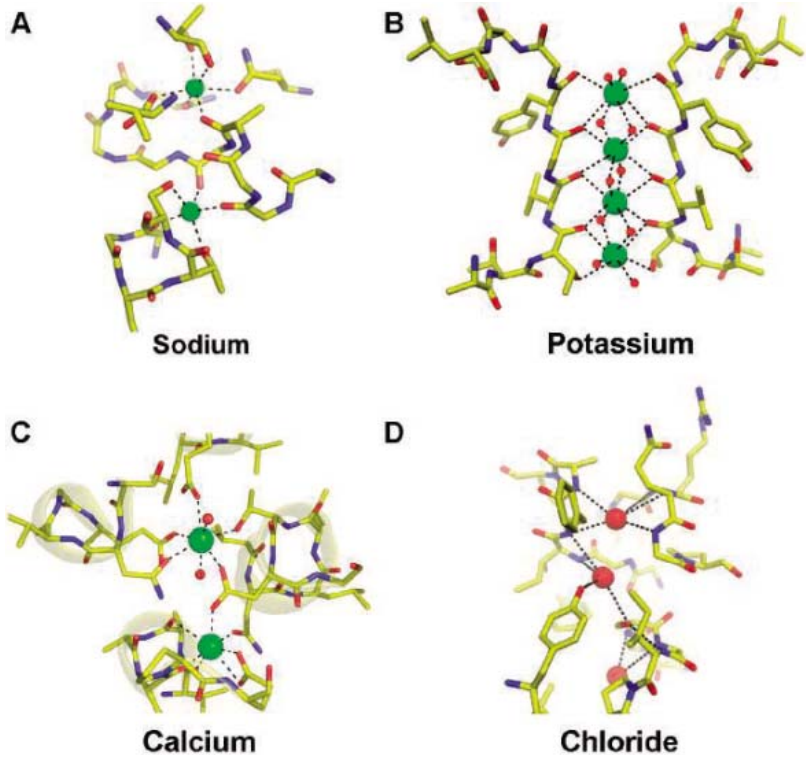

Fig. 3. Na^+-, K^+-, Ca^{2+}-, and Cl^--selective binding sites in transport proteins. (**A**) Two Na^+ binding sites in the LeuT Na^+-dependent pump (PDB code 2A65). (**B**) Four K^+ binding sites in the KcsA K^+ channel (PDB code 1K4C). (**C**) Two Ca^{2+} binding sites in the Ca^{2+} ATPase pump (PDB code 1EUL). (**D**) Two central Cl^- binding sites in a mutant ClC Cl^-/H^+ exchanger (PDB code 1OTU).

K^+ channels conduct K^+ ions selectively across the cell membrane, down the electrochemical gradient. The K^+ channel contains four K^+ binding sites in a row, forming a selectivity filter (Fig. 3B) (*3*, *14*). In each of these sites, a K^+ ion is dehydrated and interacts with eight partial-charge–bearing oxygen atoms (main-chain carbonyl or side-chain hydroxyl atoms). The size of the cavity formed by the selectivity filter sites is a good match to the K^+ ion, with a mean K^+-O distance of 2.84 Å. The greater number of oxygen atoms forming the K^+ binding sites (eight oxygen atoms) compared to that of the Na^+ sites (five or six oxygen atoms) is a simple geometric consequence of the larger radius of K^+, which allows a greater number of oxygen atoms to surround the ion (*10*, *15*, *16*).

What does comparison of LeuT and the K^+ channel teach us about alkali metal ion selectivity in transport proteins? The Na^+ and K^+ sites both contain oxygen atoms, mostly the kind with partial negative charges. This agrees well with the rules learned from host/guest chemistry with ions. There is a tendency for Na^+ sites to contain one formal charge, undoubtedly owing to this ion's smaller radius and higher charge density, but a formal charge is not essential for a Na^+-selective site (*10*, *15*). A very important factor distinguishing Na^+ and K^+ sites is the size of the cavity formed by the binding site. This also agrees with the small-molecule chemical literature. Chemists have created molecules of a given class with selectivity favoring Li^+ (radius 0.60 Å), Na^+ (radius 0.95 Å), K^+ (radius 1.33 Å), or Rb^+ (radius 1.48 Å) by simply adjusting the cavity size to match the ion (*10*). LeuT and the K^+ channel suggest that the essence of alkali metal cation selectivity is similar to that in ion-binding small molecules: The protein selects for a particular ion, Na^+ or K^+, by providing an oxygen-lined binding site of the appropriate cavity size.

Size selectivity does not mean that an ion binding site has to be rigid. A rigid, preformed, binding-site cavity can increase its affinity for an ion through entropic terms in the free energy (*10*), but it is not a requirement for size selectivity. Size selectivity comes about through the potential of a molecular structure to conform more favorably to an ion of a particular size. Beautiful examples of this are provided by nonactin and valinomycin, which are molecular cages that bind K^+ selectively over Na^+ (*17*, *18*). The structure of these molecules changes depending on whether the binding site is empty or occupied by K^+ or Na^+ (*17*, *18*). These molecules are not rigid, but we can nevertheless understand their selectivity in terms of the energetic stability of the K^+ complexes because of the shapes and sizes they are able to adopt. Nonactin and valinomycin more com-

fortably (that is, without strain) fit around a K^+ ion and so more effectively compensate for the energy cost of K^+ ion dehydration. Indeed, the K^+ channel in this respect is quite similar to, but perhaps even more extreme than, the small K^+-selective molecules. In crystal structures, when Na^+ replaces K^+ in solution, the selectivity filter of the K^+ channel undergoes a conformational change and collapses shut, occluding two of the four binding sites (Fig. 4A) (*14*, *19*). In other words, the selectivity filter sites in a K^+ channel comfortably fit around K^+ ions and not Na^+ ions when the filter adopts what we recognize as a conductive conformation. This is an elegant example of size selectivity, and it has little to do with the rigidity of the ion binding sites. In fact, one could say that the selectivity for conducting K^+ ions is enabled by the ability of the protein to undergo particular conformational changes and thus form the correct coordination sphere for K^+ in response to the presence of K^+.

The importance of a connection between protein conformation and ion binding site selectivity (and affinity) is even more obvious in the case of ion pumps, in which the properties of ion binding are coupled to conformational states of the protein in an obligatory manner in order to move ions against their electrochemical gradient (the Ca^{2+} ATPase), or to allow ion gradients to move another substrate against its gradient (LeuT).

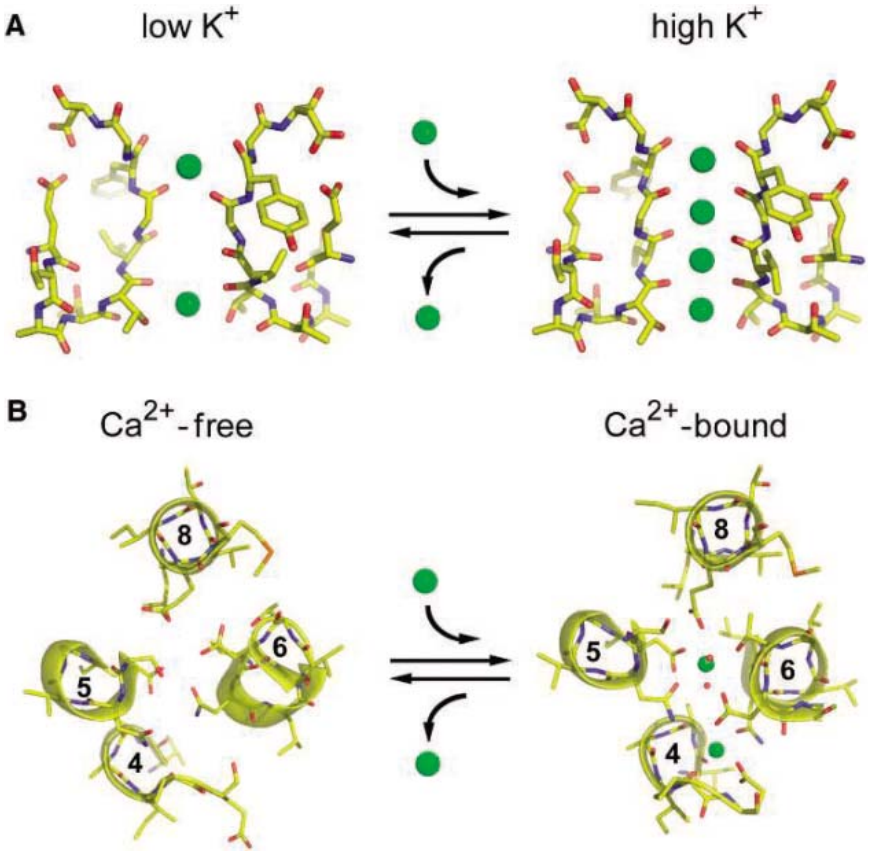

Fig. 4. Ion binding correlated with protein conformational changes in a K^+ channel and a Ca^{2+} pump. (**A**) The selectivity filter of a K^+ channel in the presence of low K^+ concentrations (high Na^+) undergoes a conformational change that occludes its two innermost binding sites (left, PDB code 1K4D). K^+ entry at high K^+ concentrations induces the conductive filter conformation, which contains four sequential K^+ binding sites (right, PDB code 1K4C). (**B**) Ion binding loci in a Ca^{2+}-free conformation of the Ca^{2+} ATPase (left, PDB code 1IWO) undergo conformational changes to form a Ca^{2+}-bound conformation (right, PDB code 1EUL). Helix numbers are shown.

Calcium

The Ca^{2+} ATPase pump has to discriminate between Ca^{2+} and the other dominant cations in biology: Na^+, K^+, and Mg^{2+} (*20*). Atomic structures in numerous conformations have revealed how this pump creates high-affinity Ca^{2+} binding sites in one conformation and then reorganizes the sites in another to change their Ca^{2+} affinity (*21*, *22*). In the high-affinity conformation, Ca^{2+} ions bind at two sites wedged between α helices inside the membrane (Fig. 3C). One site is formed out of oxygen atoms from side chains and water molecules, and the other is formed out of side- and main-chain oxygen atoms. The main-chain oxygen atoms are made available because one of the α helices is disrupted inside the membrane, freeing the carbonyl oxygen atoms from their usual hydrogen-bonding interactions. Seven oxygen atoms surround the Ca^{2+} ion in both sites. An obvious difference between the Ca^{2+} binding sites and the Na^+ and K^+ sites discussed above is the greater importance of fully charged oxygen atoms contributed by glutamate and aspartate side chains: A higher charge density is apparently required to compensate for the dehydration of a divalent cation. Because Ca^{2+} is such an important regulatory ion inside cells, there are many examples of proteins with Ca^{2+} binding sites (*23*). A coordination number of seven is very common, just as is observed in the Ca^{2+} ATPase sites, and we can understand selectivity for Ca^{2+} over Mg^{2+} at least in part because Mg^{2+} prefers a coordination number of six (*24*).

In the Ca^{2+}-free conformation, the α helices within the membrane undergo structural rearrangements that disrupt the Ca^{2+} binding sites by shifting the positions of the coordinating oxygen atoms (Fig. 4B) (*22*). The Ca^{2+} ATPase provides a very good example of what must be a general property of transport proteins: the ability to form selective ion binding sites in a protein conformation–dependent manner.

Chloride

The Cl^- ion is the only halogen ion used in abundance in biological systems. Br^- and I^- are used in specialized circumstances, but their abundance is low. Thus, Cl^- transport proteins seem to be faced with the modest challenge of selecting Cl^- over phosphate, sulfate, bicarbonate, and anionic proteins. Crystal structures of two prokaryotic members of the ClC Cl^- channel/Cl^--H^+ exchanger family provide a first view of the chemistry underlying Cl^- selectivity in transport proteins (*4*, *5*). These proteins blur the distinction between channels and pumps: Some members of the ClC family are channels (such as ClC-0 from the torpedo fish) (*25*) whereas others are Cl^-/ H^+ exchangers (such as ClC from *Escherichia coli*) (*26*), and yet their related amino acid sequences indicate that they have essentially the same protein structure. Apparently, subtle properties of the ion pathway distinguish the channel and pump members of this family.

The ClC transport proteins have a Cl^- selectivity filter located at the neck of an hourglass-shaped ion pathway that forms a row of Cl^- ion binding sites near the center of the membrane (Fig. 3D) (*4*, *5*). The resolution of the ClC structures is not as high as for the Na^+ and K^+ transport proteins, but the basic chemistry of the binding sites is defined. In the narrowest region of the filter, chemical groups that surround two Cl^- ions share their proton to stabilize the ion's negative charge: The hydroxyl from a tyrosine side chain readily shares its proton because the aromatic ring stabilizes the excess negative charge left

on the oxygen atom; the serine hydroxyl, because it is hydrogen-bonded through its lone electron pair to a main-chain amide, also shares its proton readily; and finally, main-chain amide groups direct their proton toward Cl^-. This selectivity filter does not distinguish between Cl^- and Br^- with high fidelity (*27*), but in nature such discrimination is not required. Selectivity in the ClC transport proteins can thus be understood in simple terms: The anion is stabilized by partial positive charges, and the filter is wide enough to permit Cl^- but not larger competing anions.

The Relationship Between Channels and Pumps

In our considerations of ion selectivity, we have grouped channels and pumps as a single collection of transport proteins because the chemical principles of ion discrimination are likely to be the same in both. But in our consideration of some of the architectural principles of transport proteins, we highlighted several unique structural aspects—protein shapes—that are related to the need for channels to conduct ions rapidly and for pumps to move ions against an electrochemical gradient. These different requirements of channels and pumps must influence certain aspects of the ion-binding sites, in particular their number and disposition in the transport protein. We observe in the K^+ channel a queue of four K^+ binding sites spanning a distance of 12 Å, which then opens into wide aqueous regions on both ends. This arrangement of ion-binding sites makes sense for rapid conduction because it provides the shortest path for diffusion across the membrane and for repulsion between adjacent ions in the queue. We observe in the LeuT and Ca^{2+} ATPase pump proteins something very different: ions embedded deep inside the protein with no unobstructed pathway to the aqueous surfaces. This arrangement makes sense for proteins that can move an ion against its concentration gradient (using the energy of ATP hydrolysis or a chemical gradient for leucine), because what is most important for a pump is not speed, but the property that it will never open its ion pathway to both sides of the membrane at the same time. A crystal structure of a pump should thus under most circumstances show buried ions. The ClC proteins, on the other hand, seem to be an intermediate class of transport proteins in between pumps and channels. Structurally, they exhibit channel-like features, including vestibules leading to a short selectivity filter with a queue of ions. Functionally, some are true channels whereas others are pumps driven by the countertransport of protons. Perhaps as the collection of ion-transport proteins grows, we might expect to see a continuum ranging from the channels to the pumps.

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Evidence for a One-Allele Assortative Mating Locus

Daniel Ortíz-Barrientos*† and Mohamed A. F. Noor‡

For decades, evolutionary biologists strove to understand how, or even if, the process of speciation can be completed despite gene flow between the emerging species (*1*). Homogenization via gene flow should necessarily impede divergence and subsequent speciation. However, this proposed antagonism between gene flow and divergence is reduced if speciation occurs through the spread of the same assortative mating allele in both of two emerging species (a "one-allele mechanism") (*2*). For example, if an allele that specifically causes dispersal reduction or increased pheromone sensitivity spreads into the diverging populations, it may reduce subsequent hybridization and gene flow and thus help to complete speciation (*3*).

We directly tested for a one-allele assortative mating mechanism in the fruit fly *Drosophila pseudoobscura*. Females from populations of *D. pseudoobscura* that co-occur with its sibling species, *D. persimilis*, exhibit high reluctance to mate with *D. persimilis* males. In contrast, females from disjunct populations are more inclined to hybridize. We previously mapped the genetic basis of this behavioral difference among *D. pseudoobscura* populations to two chromosomal regions, including one on the fourth chromosome called *Coy-2* (*4*). By using serial backcrosses, we performed eight introgressions of the *Coy-2* region from *D. pseudoobscura* into *D. persimilis* (*5*). Four of the *Coy-2* introgressions originated from a *D. pseudoobscura* population that co-occurs with *D. persimilis* (Mather, California; hereafter called perCOYsym), and the remaining four originated from a disjunct population (Flagstaff, Arizona; perCOYallo). Our procedure purged the introgression-line genomes of all *D. pseudoobscura* segments not on the chromosome bearing the introgressed *D. pseudoobscura Coy-2* alleles, as well as much of chromosome 4 outside of the *Coy-2* region. Hence, the final lines were largely pure *D. persimilis*, aside from a homozygous copy of the introgressed *D. pseudoobscura Coy-2* region. Females from perCOYsym and perCOYallo lines were then individually paired with *D. pseudoobscura* males, and their matings in 10 min were recorded. PerCOYsym and perCOYallo lines were randomly paired for this comparison to control for environmental effects on mating propensity.

If *Coy-2* behaves as a one-allele assortative mating locus, then females from perCOYsym lines should be more reluctant than females from perCOYallo lines to mate with *D. pseudoobscura* males. Hence, the same allele would confer greater assortative mating in both a *D. pseudoobscura* and a *D. persimilis* genetic background. Our results confirmed this prediction; in each comparison, females from the perCOYsym line mated less than perCOYallo females did with *D. pseudoobscura* males (Table 1). We also tested for various confounding factors. First, *D. pseudoobscura* males were indiscriminate in their courtship of perCOYallo versus perCOYsym females [all males courted continually; mean latency perCOYallo: 49.1 s; mean latency perCOYsym: 44.9 s, not significant (NS)], suggesting that the difference resulted from the behavior of the females. Second, the behaviors of the perCOYsym and perCOYallo females were identical when paired with *D. persimilis* males; all mated and did so with the same copulation latency (perCOYallo: 10.2 s; perCOYsym: 9.8s; NS). This result suggests that the difference was in female species discrimination (assortative mating) rather than a general reluctance to mate by perCOYsym females.

Did *Coy-2* spread between these two species and reduce their subsequent hybridization? If this spread occurred as a one-allele assortative mating mechanism, then we predict that *D. persimilis* females from the original strain to which we introgressed *Coy-2* should exhibit assortative mating most similar to perCOYsym females. We confirmed this prediction in a separate experiment; similar proportions of *D. persimilis* (7 of 100) and perCOYsym (8 of 100) mated with *D. pseudoobscura* males, whereas perCOYallo females mated more readily (25 of 100) (Fisher's exact value < 0.002). Further, the one-allele model predicts that there should be molecular evidence for introgression in the vicinity of *Coy-2*. Machado *et al*. studied one locus within 500 kilobases of *Coy-2* (*DPS4003*) and documented "the largest numbers of shared polymorphisms and the highest estimates of population migration rate between *D. pseudoobscura* and *D. persimilis*," (*6*) confirming our second prediction. We could not perform the reciprocal genetic introgression test because all populations of *D. persimilis* co-occur with *D. pseudoobscura*, but every line of evidence at hand strongly indicates that *Coy-2* evolved as a one-allele assortative mating locus in *D. pseudoobscura* and *D. persimilis*.

Many theoretical models have shown that one-allele assortative mating mechanisms facilitate controversial modes of speciation such as sympatric speciation and reinforcement (*3*). Such mechanisms have been inferred from phenotypes such as imprinting on paternal song characteristics or homing to one's natal habitat, but this study provides direct genetic evidence. This finding suggests that the type of variation most conducive to modes of speciation invoking gene flow during some part of the divergence process exists in nature.

Table 1. Percentage of *D. persimilis* introgression-line females (four replicates) that mated with *D. pseudoobscura*.

***D. persimilis* females**	**One-allele model prediction**	**Number (of 100) mated with *D. pseudoobscura* males**			
		rep1	**rep2**	**rep3**	**rep4**
perCOYsym	Discriminant	10	5	7	10
perCOYallo	Less discriminant	24	16	17	22

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Radiocarbon Variability in the Western North Atlantic During the Last Deglaciation

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We present a detailed history of glacial to Holocene radiocarbon in the deep western North Atlantic from deep-sea corals and paired benthic-planktonic foraminifera. The deglaciation is marked by switches between radiocarbon-enriched and -depleted waters, leading to large radiocarbon gradients in the water column. These changes played an important role in modulating atmospheric radiocarbon. The deep-ocean record supports the notion of a bipolar seesaw with increased Northern-source deep-water formation linked to Northern Hemisphere warming and the reverse. In contrast, the more frequent radiocarbon variations in the intermediate/deep ocean are associated with roughly synchronous changes at the poles.

The last deglaciation was punctuated by numerous distinct millennial-scale climate events (*1*, *2*), and understanding the mechanisms behind these changes is a major goal of paleoceanography. The deep ocean stores and transports heat and carbon, so changes in its circulation are likely to influence global climate. Indeed, alternating the main site of deep-water formation between the Northern and Southern hemispheres has been linked to switches in the amount of cross-equatorial heat transport (*3*). This bipolar seesaw predicts sizable changes in mass transport in the deep North Atlantic and may be the cause of anti-phase warm and cool periods observed in Greenland and Antarctic ice cores during the last deglaciation (*1*, *2*) (Fig. 1). Well-dated high-resolution records are needed to make a mechanistic connection between deep-ocean circulation and climate. Passive geochemical tracers from marine sediments show us that during the last glacial maximum (LGM), Northern-source water (NSW) overlay Southern-source water (SSW), with the boundary at ~2,000 m in the western North Atlantic (*4*, *5*). The transition from the LGM to the modern state, where North Atlantic Deep Water (NADW) dominates the Western basin, was marked by a series of changes in the deep-ocean circulation pattern (*6*, *7*). To help characterize those changes more completely, we have made $^{14}C/^{12}C$ measurements of well-dated samples of the deep-sea coral *Desmophyllum dianthus* (*8*).

A radiocarbon age can be deduced for a given water mass if its radiocarbon content (^{14}C 5730 year half-life) is known both when it forms and when it reaches the deep ocean. By making depth profiles of $\Delta^{14}C$ in the past (*9*), we can investigate variability in deep-ocean $\Delta^{14}C$ values and begin to put constraints on changes in ocean circulation. Deep-sea radiocarbon records can also be used to investigate the role of the ocean in modulating the atmospheric carbon reservoir. The ocean contains ~60 times as much carbon as the atmosphere, so small changes in uptake or release of radiocarbon from the ocean may cause large changes in atmospheric $\Delta^{14}C$. Today, radiocarbon-enriched NADW formation draws down atmospheric ^{14}C more efficiently than radiocarbon-depleted Antarctic Bottom Water (AABW) formation, so varying the proportion of NSW to SSW or changing the flux of NSW are both likely to change atmospheric $\Delta^{14}C$. Our record of ocean $\Delta^{14}C$ lets us constrain the influence of the deep ocean on atmospheric radiocarbon.

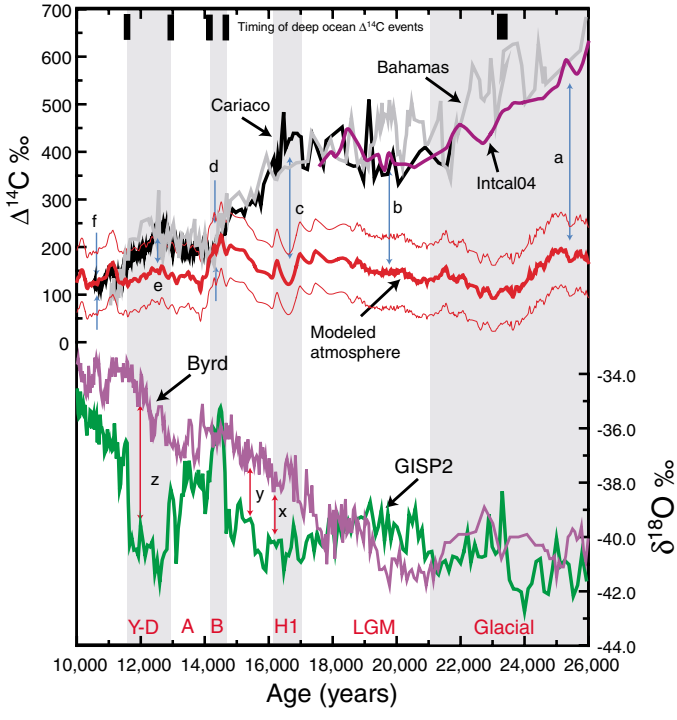

Fig. 1. The top three curves are observed atmospheric $\Delta^{14}C$ records: Cariaco Basin in black (*34*), a Bahamas speleothem in gray (*35*), and Intcal04 in purple (*39*), which, during the main period of interest, is primarily based on precisely dated surface coral data (*53*). All of these records are in reasonable agreement from 10 ka back to ~15.5 ka, but there are differences during Heinrich 1. The age model for the Cariaco Basin is poorly constrained during Heinrich 1, with the exception of a distinct change in gray-scale that matches a $\delta^{18}O$ event in both GISP2 and a U-Th dated speleothem from Hulu Cave (*54*) at 16.0 ka. Two outliers have been removed from the Cariaco record (16.10 ka and 17.96 ka). Between 26 and 22 ka, we plot only the Intcal04 (*39*) and speleothem data (*35*), which are consistent with one another. When comparing our ocean $\Delta^{14}C$ data to the atmosphere, we refer to Intcal04 (*39*) except between 17.5 ka and 14.5 ka, where there are no surface coral data and the record is poorly constrained. In this 3000-year period, we combine the Cariaco (*34*) and speleothem records (*35*) as our best estimate of the atmosphere. The ^{10}Be-based $\Delta^{14}C$ (modeled) reconstruction is plotted on the same scale as the observed atmospheric record, with the maximum and minimum as thin lines and the mean as a thick red line (*36*). Arrows a to f and x to z point to times that are referred to in the text. The GISP2 (green) and Byrd $\delta^{18}O$ (purple) records are plotted after Blunier and Brook (*2*).

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Using radiocarbon as a circulation tracer has been successful in the modern ocean. NADW and AABW have end-member values of –65‰ (per mil) and –165‰, respectively (*10*, *11*). Radioactive decay causes deviations below the mixing line of these two end members, allowing us to calculate the radiocarbon age of the water in the North Atlantic (*12*). In the modern western North Atlantic [GEOSECS (Geochemical Ocean Section Study) Station 120, 33°16′N], the water column has a small vertical $\Delta^{14}C$ gradient (~10‰ /1000 m) consistent with a single northern-source water mass (*13*). By contrast, farther south in the Atlantic, NADW is underlain by southern-sourced AABW. AABW has a characteristic low $\Delta^{14}C$ because the "old" Pacific intermediate water from which it forms is not at the surface long enough to reequilibrate with the atmosphere. In the past, this approach is complicated by variability in the two end-member $\Delta^{14}C$ values at the sites of deep-water formation (*14*). For example, increasing the extent of sea-ice cover would allow less air-sea gas exchange and, therefore, less radiocarbon in AABW. Constraints on the past deep-ocean $\Delta^{14}C$ have been acquired using the radiocarbon ages of benthic and planktonic foraminifera (BF-PF) and the aragonitic skeletons of deep-sea corals. In the foraminifera, the planktonic age can be converted to a calendar age, and the benthic $^{14}C/^{12}C$ ratio can then be used to calculate deep-ocean $\Delta^{14}C$. Early $\Delta^{14}C$ reconstructions suffered from problems of species-dependent age variability in planktonic $^{14}C/^{12}C$ measurements (*15*–*18*), but this problem is alleviated by targeting depths with high foraminiferal abundances or high sedimentation rates (*19*).

Deep-sea corals, typically found at water depths of ~500 to 2500 m, are datable by U-Th techniques and are good archives of palaeo-$\Delta^{14}C$ (*20*–*24*). Individual corals with different calendar ages can be compared with one another to give a resolution similar to that of ocean sediment cores. The solitary coral *D. dianthus* is thought to have a life span of ~100 years (*25*), so each individual skeleton can be subsampled for $^{14}C/^{12}C$ to construct decadal-resolution records of radiocarbon variability, comparable to the temporal resolution of ice-core climate records (*20*). We collected more than 3700 *D. dianthus* corals from the New England Seamounts in May 2003 (*26*). U-Th isotopic measurements were made by isotope dilution, and 27 samples were selected for $^{14}C/^{12}C$ analysis (*27*) (table S1). Nine of these corals were subsampled to produce high-resolution transects of $\Delta^{14}C$. Our second sample set consists of 12 BF-PF pairs [spanning 19.5 thousand years ago (ka) to 10.7 ka] from the western North Atlantic and one additional sample using a benthic bivalve found in the core (table S2). Combining these data with published deep-sea corals and BF-PF pairs (*19*, *20*, *28*–*32*) allows us to reconstruct a detailed history of radiocarbon from the last glacial through to the Holocene.

Discussion. The ^{14}C content of the atmosphere and the deep sea are coupled, but our knowledge of the history of these two reservoirs is vastly different. The history of radiocarbon variability in the atmosphere is reasonably well constrained through the LGM and beyond (*33*–*35*). Radiocarbon and ^{10}Be are produced in the upper atmosphere simultaneously, and because ^{10}Be is not subject to decay or uptake in the carbon cycle, it can be used as a proxy for the ^{14}C production rate alone (*36*). Muscheler *et al*. (*36*) estimate the ^{10}Be production rate from the measured ^{10}Be content of Greenland ice cores and convert it to an expected atmospheric $\Delta^{14}C$ record, assuming that the present-day carbon cycle was the same throughout (hereafter referred to as modeled atmospheric $\Delta^{14}C$) (Fig. 1) (*37*, *38*). Changes in observed atmospheric $\Delta^{14}C$ that are greater than predicted from the production rate curve alone must be due to deviations from the assumed modern steady state, implying changes in the deep-ocean uptake on this 10^3- to 10^4-year time scale (*34*–*36*).

Compared with the atmosphere, our knowledge of the deep ocean is more limited, both in depth and in time. Our data fill this knowledge gap, allowing us to compare $\Delta^{14}C$ at depth intervals in the western North Atlantic directly with the changes observed in the atmosphere (Fig. 2). A contour plot of the history of oceanic radiocarbon relative to atmospheric radiocarbon provides a true-age chronology through the glacial and deglacial periods (Fig. 3). It is clear from this plot that intermediate-deep (I/D) (1700 to 2500 m) waters are more variable than the abyss. A horizontal $\Delta^{14}C$ divide separates water masses above and below ~2,500 m, and NSW penetrates below this divide only twice during the deglaciation. A deglacial,

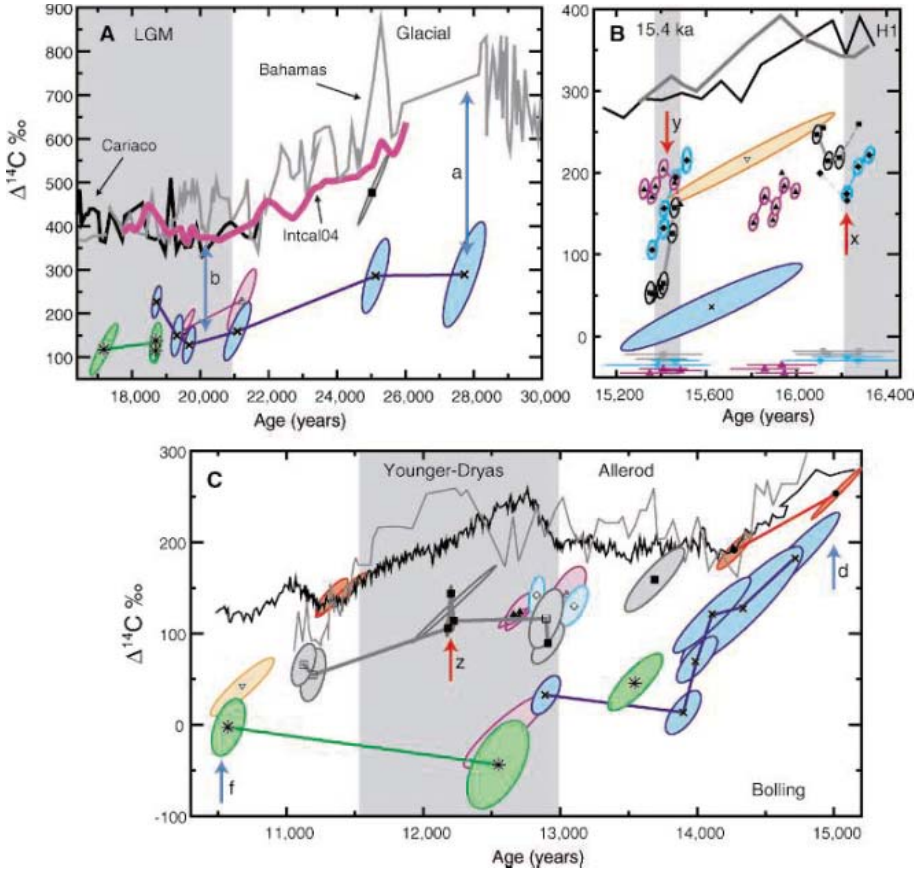

Fig. 2. Raw $\Delta^{14}C$ data for (**A**) 30.0 to 16.4 ka, (**B**) 16.4 to 15.1 ka, and (**C**) 15.1 to 10.0 ka, compartmentalized into seven depth bands: 1176 to 1221 m (red circles), 1381 to 1400 m (orange upside-down triangles), 1713 to 1790 m (black squares), 1886 to 2155 m (pale blue diamonds), 2228 to 2590 m (purple triangles), 2972 to 3845 m (blue crosses), and 4055 to 4712 m (green stars) below sea level. Each record is plotted against calendar age before the present (B.P.), with 2-SE error ellipses. Open symbols, crosses, and stars are BF-PF pairs; closed symbols are corals. One published Younger Dryas BF-PF data point (*19*) is not plotted because it lies above the atmospheric curve. In Fig. 2B, color-coded 2-SE calendar-age error bars are shown as horizontal bars, with error ellipses representing the relative error between individual points on the same coral. Solid lines join $\Delta^{14}C$ measurements from within one coral; dashed lines connect data from separate corals. Two coral data points (1886 m, 16.1 ka and 2500 m, 15.9 ka) are not shown because they have large calendar-age errors that overlap other data points (242 and 297 years, respectively). The two points with large error ellipses that are shown are BF-PF pairs.

radiocarbon-depleted I/D water mass (akin to modern Antarctic Intermediate Water) is present at 40°N during Heinrich 1, the 15.4-ka event, and the Younger Dryas.

Glacial Ocean. From 28 to 17 ka, the $\Delta^{14}C$ of the western North Atlantic is constrained by 12 points (11 BF-PF pairs and 1 coral) (Fig. 2A). The 25-ka coral at 1700 m has a $\Delta^{14}C$ only 66‰ lower than the atmosphere, indicative of well-ventilated NSW. By contrast, all deeper samples are more depleted in radiocarbon than anywhere in the modern ocean. This depletion is >400‰ at 28 ka and drops to ~230‰ during the LGM (Fig. 2A, arrows a and b, and Fig. 4A). A slowdown in the ventilation rate or a change in the proportion of NSW to SSW may account for some of this observed $\Delta^{14}C$ shift in the deep ocean. However, Cd/Ca ratios show us that the LGM deep North Atlantic was filled by SSW (*6*), and the pattern of deep-sea $\Delta^{14}C$ from 21 to 18.7 ka reflects the atmosphere nearly synchronously, suggesting that this SSW water was circulating vigorously (*39*) (Fig. 2A). An alternative cause of radiocarbon depletion in the Southern Ocean is extensive sea-ice coverage, which would reduce the amount of the air-sea carbon exchange. In support of this mechanism, diatom-based reconstructions show that the LGM sea-ice extent was 5° farther north than in the present day and that before the LGM, the sea ice exhibited less seasonal variability (*40–42*). Over the same time period, from 30 ka to the LGM, the observed and modeled $\Delta^{14}C$ of the atmospheric records converged from a large offset of >350‰ to ~250‰ (Fig. 1, arrows a and b). We suggest that the reduction in the atmospheric ^{14}C content was caused by an increase in the ^{14}C uptake in the Southern Ocean. Not only would such a change in the Southern Ocean affect the $\Delta^{14}C$ of North Atlantic water but, as the dominant source to the deep Pacific, it could alter the whole ocean ^{14}C inventory.

Heinrich 1 and the 15.4-ka event. Heinrich events are characterized by massive ice-rafted debris (IRD) deposits in the North Atlantic (*43*), and it has been suggested that these large freshwater inputs in the Northern Hemisphere may reduce the rate of northern-sourced deep-water formation by lowering the density of surface water. The Pa/Th ratio of a marine sediment record from 4500 m at the Bermuda Rise shifts toward values indicative of such a reduction during Heinrich 1 (*44*). As expected, this slowdown in NSW flux is consistent with a reduction in the amount of uptake of ^{14}C by the deep Atlantic and a divergence in the modeled and observed atmospheric records (Fig. 1, arrow c), although the signal is not large.

During Heinrich 1, the $\Delta^{14}C$ water-column profile is characterized by radiocarbon-rich water overlying radiocarbon-poor water (Fig. 4A), indicative of a greater proportion of SSW deeper in the water column. This deep water in the North Atlantic has the same offset from the atmosphere as the southern-source end member (~265‰, constrained by a 16.7-ka deep-sea coral from the Drake Passage) (*21*), which implies a vigorous deep SSW circulation. These radiocarbon data are not consistent with the Bermuda Rise Pa/Th record (*44*) if the latter is interpreted as a dramatic reduction of deep-water ventilation rate. On the other hand, our data show the $\Delta^{14}C$ of I/D water decreasing through Heinrich 1, consistent with a reduction in NSW flux (Fig. 2B). Six coral individuals describe a "U-shaped" change in $\Delta^{14}C$ in the I/D ocean beginning at 16.3 ka (Fig. 2B, arrow x). Multiple measurements from within one coral at 2,000 m define a $\Delta^{14}C$ decrease of 50‰ in ~100 years (Fig. 2B). This decrease occurred faster than the rate of ^{14}C decay, so it must be due, at least in part, to mixing-in of low $\Delta^{14}C$ SSW. This downward trend reverses at 16.2 ka, when multiple ^{14}C measurements within a second coral define a 30‰ rise in $\Delta^{14}C$ caused by an increase in the influence of radiocarbon-rich NSW (Fig. 2B). The timing of the turn in the $\Delta^{14}C$-U-shape, 16.2 ka, is coincident with the start of the dramatic decrease in $\Delta^{14}C$ observed in the atmosphere (within calendar-age error limits) and signals the end of Heinrich 1 at I/D depths.

After Heinrich 1, at 15.5 ka, all corals from 1700 to 2500 m have a $\Delta^{14}C$ signal ~100‰ lower than the atmosphere, indicative of a

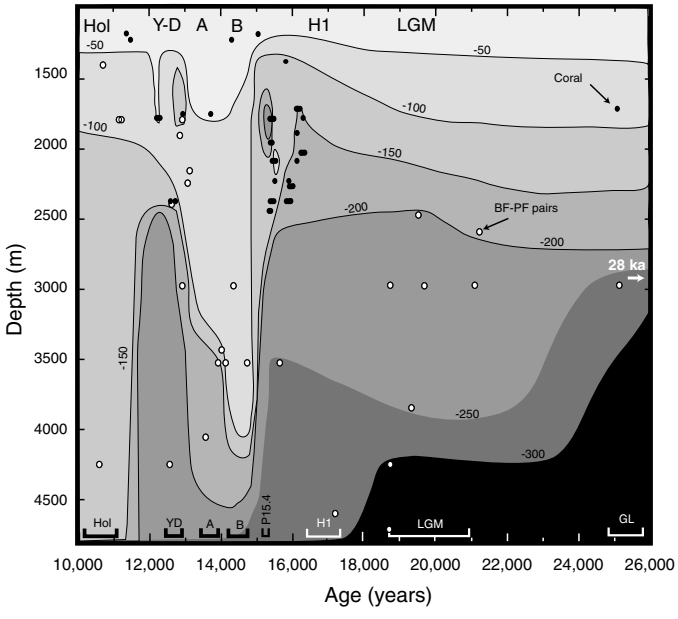

Fig. 3. Cartoon contour plot for all coral (closed circles) and BF-PF (open circles) data from 26 to 10 ka and deeper than 1000 m in the western North Atlantic. Data are plotted relative to the atmospheric record shown in Fig. 1. Dark colors are low in radiocarbon, and light colors are rich in radiocarbon. One BF-PF sample from 27.7 ka (*19*) lies off the time axis; it is from 2972 m and has a >400‰ offset from the atmosphere. Brackets at the bottom show the time intervals for the depth profiles in Fig. 4.

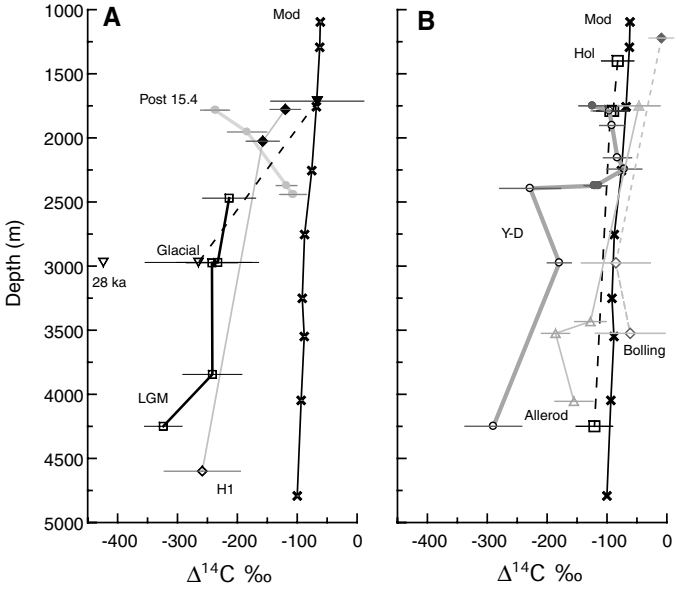

Fig. 4. Depth profiles of $\Delta^{14}C$ relative to the atmosphere in the ocean are chosen from discrete time slices (**A**) from the glacial to 15.4 ka and (**B**) from the Bolling to the Younger Dryas. Modern GEOSECS water column data are shown as crosses in both panels (*13*). Open symbols are BF-PF pairs; closed symbols are corals. The data in each profile are bracketed in Fig. 3 and marked in table S3. These profiles are not representative of "steady-state" ocean configurations, but they are plotted to demonstrate the large and variable radiocarbon gradients in the deep and I/D ocean.

well-mixed, well-ventilated northern-source I/D water column. Deeper, at 3500 m, SSW still fills the deep-water column, as shown by a BF-PF pair with a 250‰ offset from the atmosphere (Fig. 2B).

The modeled and observed atmospheric $\Delta^{14}C$ records converge after Heinrich 1. This convergence is interrupted by a plateau in the atmospheric $\Delta^{14}C$ record that lasts from ~15.7 to 15.0 ka and is coincident with a pause in the deglacial temperature rise in both hemispheres (Fig. 1, arrow y). At water depths of 1700 to 2000 m, this 15.4-ka event is characterized by a massive and rapid (100‰ in ~100 years) drop in $\Delta^{14}C$ (*20*) (Fig. 2B, arrow y). Multiple ^{14}C measurements within the lifetime of each of four individual corals from two different seamounts clearly define this trend. The decrease is much faster than in situ decay of ^{14}C and must be due to mixing-in of low $\Delta^{14}C$ SSW. A $\Delta^{14}C$ decrease is also seen at 2500 m, but with a lesser amplitude (~40‰). We interpret this change as a ^{14}C-depleted front spreading northward, rather than a shoaling of deeper water, because the $\Delta^{14}C$ is lower at 2000 m than at 2500 m. This increase in the volume of I/D SSW at the expense of ^{14}C-rich NSW formation at the 15.4-ka event is the likely cause of the ^{14}C plateau in the atmosphere.

At the end of the 15.4-ka event, the water column has an ''inverted'' profile with radiocarbon-poor water overlying radiocarbon-rich water (Figs. 2B and 4A). This inverted profile is analogous to GEOSECS profiles farther south in the modern west Atlantic, although the gradients are much smaller in the modern ocean. Deglacial intermediate SSW masses have previously been observed in benthic $\delta^{13}C$ records at lower latitudes in the Tasman Sea and at the Chatham Rise (*45*, *46*), but this is the first time that water with such low $\Delta^{14}C$ is seen at I/D depths so far north.

Bolling-Allerod to Holocene. The Bolling begins at ~14.6 ka on the Greenland Ice Sheet Project 2 (GISP2) time scale (*2*, *47*) and is widely recorded across the Northern Hemisphere (Fig. 1). Cd/Ca ratios (*6*) and Nd isotopes (*48*), respectively, show that NSW dominated the deep Atlantic during the Bolling. The modeled and observed $\Delta^{14}C$ records converge with one another at this time (Fig. 1, arrow d), suggesting that the Bolling ocean was capable of drawing down as much radiocarbon as the modern carbon cycle. Consistent with this suggestion, we observe that the $\Delta^{14}C$ depth profile is more like the modern ocean than at any time since the glacial (*49*) (Fig. 4B). The entire water column is filled by radiocarbon-rich water, with deep water (3500 m) only 70‰ lower than the atmosphere (Fig. 4B). Radiocarbon-rich water also invaded the eastern Atlantic at this time (*50*). The timing of this NSW flush is consistent with the reinvigoration of export from the North Atlantic as recorded by Pa/Th (*44*).

The end of the Bolling is characterized by cooling (Fig. 1) (*2*) and is coincident with a 700-year, 160‰ drop in deep-ocean $\Delta^{14}C$ at 3500m (Fig. 2C) (*51*).The resulting Allerod water-column $\Delta^{14}C$ profile is consistent with Cd/Ca ratios, which indicate that the deep Atlantic was filled by a mix of NSW and SSW (*6*). This mixture was ~170‰ offset from the atmosphere, as shown by BF-PF pairs from 3500 m (13.9 ka) and 4250 m (13.6 ka) and an equatorial deep-sea coral from 2300 m (~14.0 ka) (*23*, *24*). This reduction in the amount of deep NSW formation is the likely cause of the divergence of modeled and atmospheric $\Delta^{14}C$ records in the transition from the Bolling to the Allerod (Fig. 1).

The beginning of the Younger Dryas, at 12.9 ka, is characterized by a large decrease in Northern Hemisphere temperature (Fig. 1). The $\Delta^{14}C$ depth profile is ''inverted'' between 1700 m and 2500 m (Fig. 4B) (*20*, *28*), but at ~2500 m there is a sharp transition back to radiocarbon-depleted deep water, ~300‰ offset from the atmosphere (Fig. 4B) (*19*). The increased proportion of radiocarbon-depleted SSW at I/D and abyssal depths is the likely cause of the marked divergence between the modeled and observed atmospheric $\Delta^{14}C$ records (Fig. 1, arrow e) (*34*, *52*). During the Younger Dryas, at ~12 ka, Eltgroth *et al.* (*28*) report a $\Delta^{14}C$ ''spike'' at I/D water depths from corals on either side of the North Atlantic [New England Seamount (Fig. 2C, arrow z) and Azores]. This transient flushing of well-ventilated NSW is synchronous with a kink in the atmospheric $\Delta^{14}C$ record and a brief warm event observed in both Antarctica and Greenland ice cores (Fig. 1, arrow z).

At the end of the Younger Dryas, four BF-PF pairs spanning 1400 m to 4250 m all have the same ~100‰ $\Delta^{14}C$ offset from the atmosphere (Fig. 3A). This final flush of radiocarbon-rich NSW fills the entire depth range of the western Atlantic by 10.6 ka, drawing down ^{14}C and causing the modeled and observed atmospheric $\Delta^{14}C$ to converge. This overall circulation pattern and associated carbon cycle is similar to the modern day (Fig. 1, arrow f).

Conclusions. The deep-ocean radiocarbon pattern supports the notion of the bipolar seesaw: When the deep ocean was flushed by radiocarbon-rich NSW, Greenland was warming, and when NSW was replaced by SSW, Greenland was cooling. The I/D ocean is much more variable, with multiple switches between radiocarbon-depleted and radiocarbon-enriched water masses (Fig. 3). These I/D-ocean radiocarbon events are associated with small climate changes observed in both Greenland and Antarctic ice cores. Increasing $\Delta^{14}C$ in the I/D ocean is associated either with no temperature change or with warming. Decreasing I/D ocean $\Delta^{14}C$ is associated with interruptions in the rise of temperature out of the LGM. This pattern is inconsistent with a bipolar seesaw link between cross-equatorial heat flux and climate change. I/D water-mass variability does not have as large an effect on climate as deep-ocean variability but may play an important role in modulating the atmospheric carbon reservoir.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/1114832/DC1
Materials and Methods
Tables S1 to S3
References and Notes

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REPORTS

Postseismic Mantle Relaxation in the Central Nevada Seismic Belt

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Holocene acceleration of deformation and postseismic relaxation are two hypotheses to explain the present-day deformation in the Central Nevada Seismic Belt (CNSB). Discriminating between these two mechanisms is critical for understanding the dynamics and seismic potential of the Basin and Range province. Interferometric synthetic aperture radar detected a broad area of uplift (2 to 3 millimeters per year) that can be explained by postseismic mantle relaxation after a sequence of large crustal earthquakes from 1915 to 1954. The results lead to a broad agreement between geologic and geodetic strain indicators and support a model of a rigid Basin and Range between the CNSB and the Wasatch fault.

Some of the largest earthquakes in North America during the 20th century were located in the Central Nevada Seismic Belt (CNSB), one of the known actively deforming areas in the Basin and Range (Fig. 1). The 1915 Pleasant Valley earthquake [seismic magnitude (M_s) 7.2 to 7.6], the 1932 Cedar Mountain earthquake (M_s 7.2), and the 1954 Rainbow Mountain–Fairview Peak–Dixie Valley earthquake sequence (four events, M_s 6.8 to 7.2, in a 6-month period) were right lateral to normal slip events, and ruptured a noncontinuous stretch of north-northeast striking range front faults ~250 km in length.

The present-day deformation across the CNSB is puzzling for two reasons: (i) The deformation rate during Holocene time is believed to be 0.5 to 1.3 mm/year (*1*–*4*), which is lower than the 2 to 4 mm/year measured by Global Positioning System (GPS) data (*5*–*7*); and (ii) GPS measurements reveal a zone of east-west contraction east of the CNSB (*5*–*9*) that is difficult to reconcile with current geodynamic models of the region, which involve east-west extension and right-lateral shear. One possible explanation for these two discrepancies is that the GPS data record not only the long-term deformation, but also transient deformation associated with viscous or viscoelastic relaxation of the lower crust or upper mantle after the last century's earthquakes (*7*, *8*, *10*). We used 8 years of interferometric synthetic aperture radar (InSAR) data to investigate ongoing deformation in the CNSB.

The SAR imagery covers a swath nearly 700 km long (seven conventional SAR frames) acquired by the European Remote Sensing Satellites ERS-1 and ERS-2 between 1992 and 2000 to investigate crustal deformation at the CNSB (*11*). InSAR measures changes in the radar line-of-sight (LOS) distance between the satellite and the surface of Earth; it is most sensitive to vertical movement and somewhat sensitive to east-west movements (*12*). A ground velocity map in LOS direction is shown in Fig. 1. The map was obtained by averaging (stacking) eight independent long-term interferograms, each spanning 4 to 7 years (Table 1). Most of the interferograms have perpendicular baselines smaller than 100 m. We used these pairs because larger baselines lead to decorrelation of the interferometric phase. We obtained the velocity map by dividing the cumulative LOS displacement of the interferograms by the cumulative interferogram period of 37 years. We assumed that uncertainties associated with the satellite orbits cause linear phase ramps across the interferogram and removed any linear trend from the data.

The resulting ground velocity map shows a bulge with LOS velocity as high as ~3 mm/year of relative motion with respect to the margin of the interferograms, centered in the epicentral area of the 1915 Pleasant Valley and 1954 Dixie Valley earthquakes. About 1 to 2 mm/year is detected in the areas of the Fairview Peak and Cedar Mountain earthquakes. The map also shows an area of subsidence in the northern part of the interferogram in the area of the Lone Tree gold mine, presumably caused by groundwater pumping in support of open-pit mining operations.

To test whether the observed phase signature is real deformation or a processing artifact, we generated another stack using eight interferograms covering shorter time periods (each <4 months, total time span ~2 years). Because no deformation is expected from such a stack, a residual signal would reveal processing, atmospheric, or orbital artifacts. To obtain comparable LOS velocities, we divided the cumulative LOS displacement of the short-term stack by the cumulative time of the long-term stack (37 years). The averaged LOS velocities based on the long-term stack (Fig. 2) show a long-wavelength signal of ~3 mm/year of LOS velocity, but the short-term stack does not show this signal. This result indicates that the

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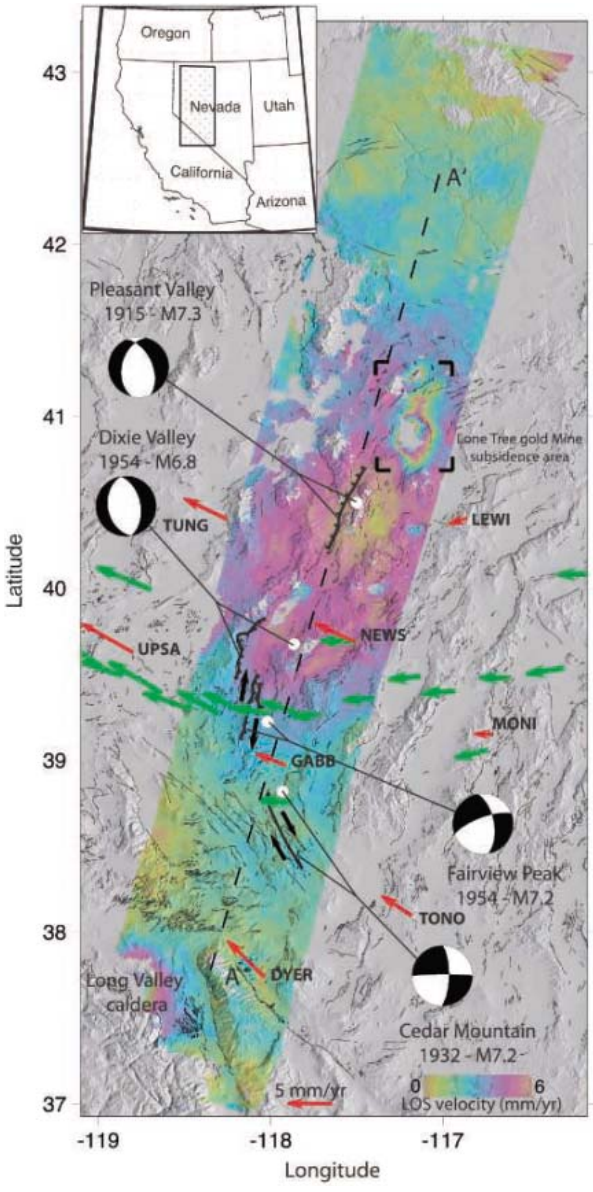

Fig. 1. 1992–2000 LOS velocity map for the area of the 1915–1954 Nevada earthquakes together with epicenters (blank circles), focal mechanisms (spheres), and surface ruptures. Green arrows, campaign GPS velocities (*7*); red arrows, Basin and Range Geodetic Network (BARGEN) permanent GPS velocities and site names (*9*). LOS is velocity considered positive for decreasing distance between ground and satellite.

Table 1. Dates of SAR acquisitions used for interferometry and perpendicular baselines.

Date range	Perpendicular baseline (m)
25 July 1992– 21 September 1999	3
1 May 1993– 10 November 1998	–41
18 September 1993– 6 October 1998	–18
16 October 1995– 23 February 1999	72
17 October 1995– 23 May 2000	–101
21 November 1995– 1 August 2000	180
9 April 1996– 5 September 2000	–71
14 May 1996– 19 May 1998	84

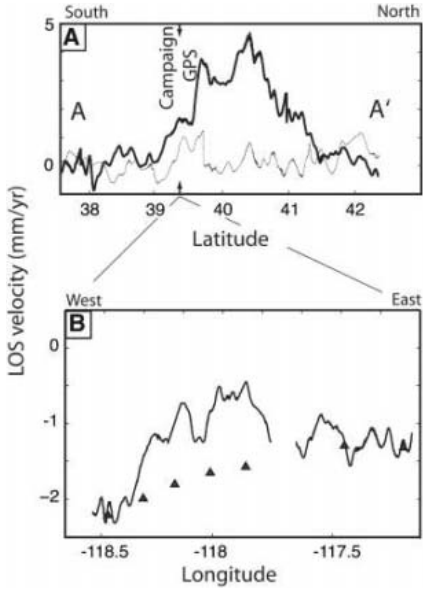

Fig. 2. (**A**) LOS velocity relative to the margins of the interferogram along a north-south profile obtained from long-term (thick line) and short-term (thin line) interferograms; the location of the GPS profile is shown by arrows. (**B**) LOS velocity relative to stable North America along a roughly east-west profile coinciding with the GPS campaign sites (line), together with LOS component of horizontal GPS velocities (triangles). The uncertainty on the LOS component of horizontal GPS velocities is on average 0.4 mm/year.

observed phase signature represents real ground deformation. Both profiles show a short-wavelength variation of 1 to 2 mm/year LOS velocity attributable to the atmospheric variability.

To test whether the InSAR data can be explained by horizontal deformation as measured by campaign GPS measurements, we compared the LOS component of the horizontal GPS with the InSAR data. The GPS data (*7*) show a roughly linear increase of ~3 mm/year in velocity magnitude westward across the interferogram. This corresponds to a decrease in LOS velocity of 0.8 mm/year (Fig. 2B) (*13*). Transferring the InSAR data into the GPS reference frame shows that the InSAR data cannot be explained by horizontal motion (Fig. 2B). The best explanation for the bulge in the InSAR data (2 mm/year at this latitude) is therefore local uplift. Analysis of the vertical velocities of the permanent GPS network indicates that the station NEWS, situated in the area of maximum LOS velocity, is moving upward with respect to the surrounding stations (*14*), in agreement with the InSAR map.

One assumption is that errors associated with satellite orbit result in linear phase ramps in the interferograms. It is also well known that orbital errors may introduce more complex large wavelength errors. We are confident, however, that the observed signal represents real deformation because LOS velocity maps based on the same SAR acquisitions but different interferograms showed similar results, because the velocity map based on short-term interferograms does not show any similar signature even though they are based on the same acquisitions, and because the detected deformation has a maximum velocity in the area of the largest historic earthquake, which is geologically plausible and consistent with GPS measurements. We attempted to verify the result with the use of data from the adjacent swath to the east, but we could not produce an interferogram with a similar cumulative time.

We tested whether the observed deformation may be caused by postseismic relaxation of Earth's crust and mantle after the 1915 to 1954 earthquakes. We assumed linear viscoelastic rheology and considered two- and three-layer Earth models using the methodol-

Table 2. Earthquake parameters used in the modeling. The magnitude values are obtained by inversion of the InSAR data and the postseismic models. See text for references.

Location and date	Latitude	Longitude	Length (km)	Depth (km)	Strike	Dip	Rake	Magnitude	
								Published (M_s)	Inversion (M_w)
Pleasant Valley (3 October 1915)	40.5	–117.5	59	9	194°	44°	–61°	7.6	7.3
Cedar Mountain (21 December 1932)	38.80	–117.98	70	13	350°	72°	–179°	7.2	7.1
Fairview Peak (16 December 1954)	39.20	–118.00	40	15	004°	60°	–150°	7.2	7.2
Dixie Valley (16 December 1954)	39.67	–117.87	42	12	008°	30°	–90°	6.8	6.7

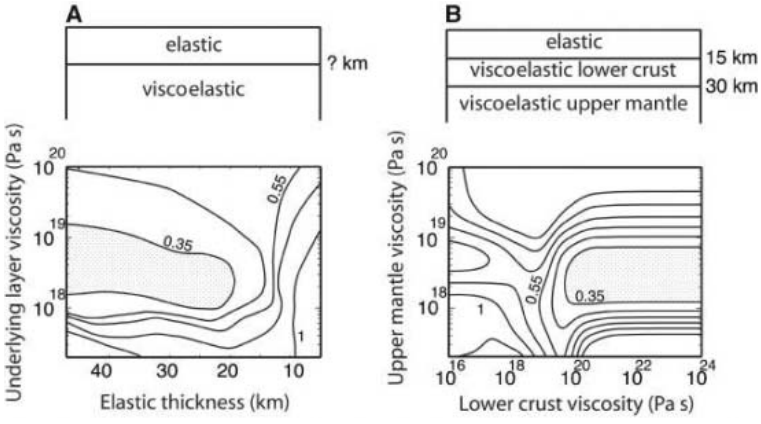

Fig. 3. Misfit between observed and modeled deformation. (**A**) Two-layer Earth model consisting of an elastic plate overlying a viscoelastic half-space. (**B**) Three-layer Earth model consisting of an elastic and a viscoelastic layer over a viscoelastic half-space.

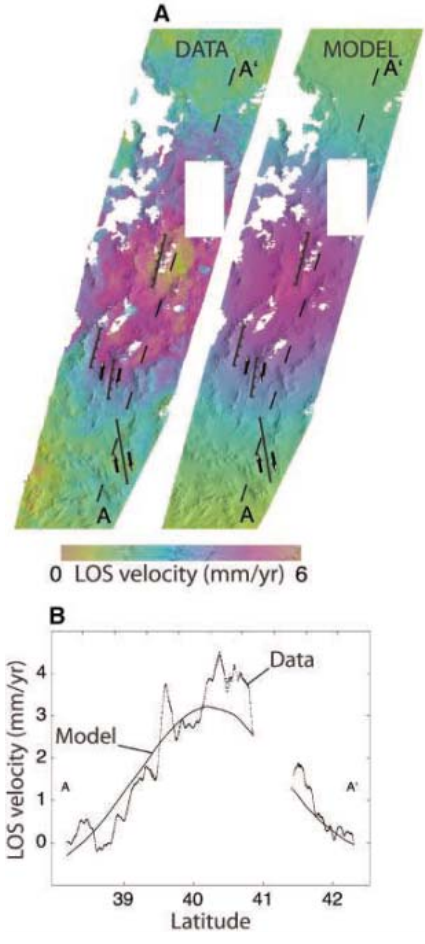

Fig. 4. (**A**) Data and best fitting postseismic relaxation model (Lone Tree gold mine deformation area has been masked out). (**B**) Profile [dashed lines in (A)].

ogy of (*15*). The sources for postseismic deformation were four earthquakes: the 1915 Pleasant Valley, 1932 Cedar Mountain, and 1954 Fairview Peak and Dixie Valley earthquakes. We did not include the main 1954 Rainbow Mountain earthquake (*16*). We used fault parameters in the range of values published after field measurements (*17*, *18*), geodetic modeling (*19*), and seismologic modeling (*20*–*22*) (Table 2). For the Dixie Valley fault we used a dip of 30° (*23*) because the maximum LOS velocity >15 km east of the surface trace of the fault suggested a low-angle dipping fault. We also inverted for the magnitudes of the earthquakes, allowing a deviation of 0.3 from the magnitudes (*21*, *22*) and from 7.1 to 7.7 for the Pleasant Valley earthquake.

Our data set consists of 11,072 equally spaced (~1.7 km spacing) LOS velocity measurements. Best fitting models are characterized by a minimum of the difference between the data and the model predictions (*24*). We varied the grid spacing and used quadtree decompositions of the data to test whether the modeling results are sensitive to the sampling method, and we found that this is not the case.

We first used a two-layer Earth model consisting of an elastic plate overlying a viscoelastic half-space to obtain an estimate of the elastic thickness of the crust and of the viscosity of the underlying substrate. We conducted a grid search varying the elastic thickness and the viscosity. For each grid point we conducted a linear inversion for the slip magnitude to account for the uncertainty of the earthquake magnitude. The lowest misfits (0.3 mm/year) were found for models with an elastic thickness larger than 20 km and a subcrustal viscosity of 10^{18} to 10^{19} Pa·s (Fig. 3).

We also used a three-layer Earth model consisting of an elastic layer overlying two viscoelastic layers, representing the elastic upper crust, the viscoelastic lower crust, and the viscoelastic upper mantle. We used an elastic layer thickness of 15 km (seismogenic thickness) and a lower crust thickness of 15 km so that the crustal thickness agreed with the 30 km inferred from seismic reflection data (*25*). We varied the viscosity of the lower crust and of the uppermost mantle. For this model the lowest misfits were found for lower crustal viscosities larger than 10^{20} Pa·s and for uppermantle viscosities of 1×10^{18} to 7×10^{18} Pa·s. The LOS velocity predicted by the best fitting model explains the large wavelength deformation (Fig. 4). We consider models with normalized root mean square (NRMS) < 0.35 mm/year as reasonable models.

The InSAR data cannot be explained with postseismic models if we use the published earthquake magnitudes, and therefore we inverted for the magnitudes. This is desirable because the magnitudes are not well constrained by the instrumental data. In fact, our study shows that precise postseismic deformation data can be used to estimate the magnitude of historic earthquakes as long as an estimate of the focal mechanism is available. We found the same magnitudes for the two- and three-layer models (Table 2). The magnitude of the Fairview peak earthquake remains unchanged at moment magnitude (M_w) 7.2. For the Cedar Mountain and Dixie Valley earthquakes, we find values of M_w 7.1 and M_w 6.7, corresponding to a reduction of 10% from the published magnitudes. For the Pleasant Valley earthquake we find M_w 7.3, smaller than the seismologic estimates of 7.6 (*26*) but in agreement with the value of 7.2 derived from surface faulting (*17*). The cumulative M_w of the four modeled earthquakes is 7.55.

It is noteworthy that the area of deformation is larger than the epicentral area of the earth-

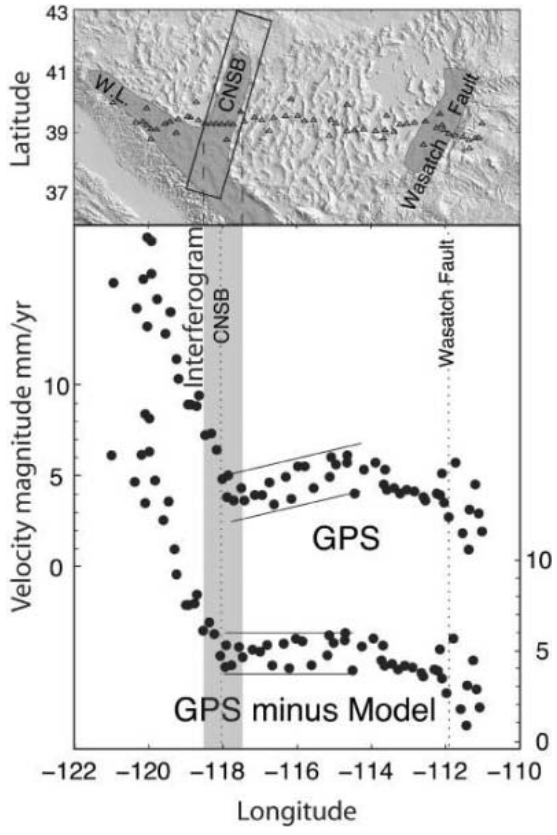

Fig. 5. Bottom: Magnitude of horizontal campaign GPS velocities along an east-west profile through the Basin and Range (*7*). Upper line, measured velocities; lower line, measured velocities minus model-predicted postseismic deformation. Top: Simplified tectonic map of the Basin and Range. Triangles, locations of GPS stations; CNSB, Central Nevada Seismic Belt; WL, Walker Lane.

quakes and that the deformation field lacks short-wavelength features. The lithospheric rheology acts as a low-pass filter that translates the instantaneous short-wavelength earthquake stress into long-wavelength deformation lasting several decades. This suggests that the upper parts of the lithosphere behave elastically on the time scale of our data and that viscous relaxation occurs only at greater depth. For the two-layer model we find a lower bound for the thickness of the elastic layer of 20 km and a viscosity of the underlying substrate of 10^{18} to 10^{19} Pa·s. Using a three-layer model, we find a viscosity of the substrate in the same range and a viscosity of the intermediate layer (lower crust) larger than 10^{20} Pa·s. These results suggest that most of the crust or the entire crust of the Basin and Range lithosphere (including the lower crust) behaved elastically for at least 80 years after these large earthquakes. Relaxation of the earthquake-induced stress occurred by viscous flow in the mantle. These rheology estimates are consistent with previous studies in the Basin and Range and in the Mojave Desert, which also showed an elastic or high-viscosity lower crust and a low-viscosity upper mantle (*27*–*31*).

The GPS data collected along an east-west profile indicate an area of low-rate contraction east of the CNSB (Fig. 5, *7*). A profile of secular ground velocity, obtained by removing the model-predicted postseismic velocities from the GPS vectors (*32*), does not show this contraction but shows only deformation west of the CNSB (Fig. 5). This suggests that the GPS-measured contraction is a postseismic effect and supports the simple geodynamic picture for the Basin and Range in which the central Basin and Range is an essentially undeforming block with deforming boundary zones (i.e., the CNSB and the Walker Lane to the east and the Wasatch fault zone to the west) (*5*, *33*, *34*). This interpretation is consistent with the geodetic microplate model for the Central Basin and Range of (*9*). The residual velocity across the CNSB itself is 0 to 2 mm/year, in agreement with geologic estimates of deformation (*4*). This implies that the CNSB does not have the elevated seismic potential attributed on the basis of the GPS measurements (*35*).

References and Notes

1. The variability of rate estimation from paleoseismologic study depends on the method used to measure the net slip vector (*2*–*4*). At the latitude of the GPS profile (at ~39.3°N), we refer to the CNSB as the area including the Rainbow Mountain, Fourmile Flat, Fairview Peak, and Sand Springs faults (*4*). This corresponds to the area between −118.646° and −117.712° between the GPS sites B220 and C200 of (*7*).
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12. The unit vector from the ground toward the radar is (0.384, –0.075, 0.92) in an [east, north, up] coordinate system for descending orbit.
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Active Microbial Sulfur Disproportionation in the Mesoproterozoic

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The environmental expression of sulfur compound disproportionation has been placed between 640 and 1050 million years ago (Ma) and linked to increases in atmospheric oxygen. These arguments have their basis in temporal changes in the magnitude of $^{34}S/^{32}S$ fractionations between sulfate and sulfide. Here, we present a Proterozoic seawater sulfate isotope record that includes the less abundant sulfur isotope ^{33}S. These measurements imply that sulfur compound disproportionation was an active part of the sulfur cycle by 1300 Ma and that progressive Earth surface oxygenation may have characterized the Mesoproterozoic.

There is a strong link between the oxidation state of the Earth's surface environment and the microbial sulfur metabolisms that influence the sulfur cycle (*1–3*). This link is revealed through sulfur isotope studies where different microbial metabolisms contributed to the final isotopic composition of sulfur species preserved in the geologic record (*4–6*). The relation between isotopic fractionation due to sulfate-reducing prokaryotes (SRP; $SO_4^{2-} \rightarrow H_2S$) and seawater sulfate concentration has been the primary tool for interpreting the sulfur isotope record of Earth surface oxidation (*7–11*). For example, the isotopic record of sedimentary sulfides reveals that SRP may have dominated the global sulfur cycle until the Neoproterozoic. After this, greater $^{34}S/^{32}S$ fractionations cannot be explained by sulfate reduction alone (*1*), and they likely reflect the added contribution of sulfur compound–disproportionating prokaryotes (SDP; $S^0/SO_3^{2-}/S_2O_3 \rightarrow SO_4^{2-} + H_2S$). Because sulfide oxidation is responsible for the intermediate sulfur compounds used by SDP (*12–15*), the widespread activity of SDP has been interpreted to indicate increased atmospheric oxygen content (*1*). New data, however, suggest that the isotopic fractionation between seawater sulfate and sulfide in the Neoproterozoic may have been smaller than previously estimated (*16*, *17*). This raises the prospect that the $\delta^{34}S$ record may not uniquely reveal the activities of SDP during the Neoproterozoic.

Recent experiments illustrated that SRP and SDP produce resolvable $^{33}S/^{32}S$ fractionations for similar magnitudes of $^{34}S/^{32}S$ fractionations (*18*, *19*). In those experiments, the compositions of sulfate associated with SDP were more ^{33}S enriched than sulfate associated with SRP (*20*). The fractionations preserved in the sulfur isotope record reflect largely the combined influence of these two metabolisms (*6*). We propose that by considering both the fractionations associated with $^{33}S/^{32}S$ and $^{34}S/^{32}S$, as preserved in ancient marine sulfide and sulfate minerals, we can elucidate the role of SRP and SDP on the global sulfur cycle. Here, we combine a steady-state, open-system isotope mass-balance model with data from sediments deposited between ~2000 and ~500 million years ago (Ma) to constrain how sulfur isotope signatures are transferred through a global sulfur cycle that includes SRP and SDP (fig. S1). The model tracks the sulfur isotopic composition of the seawater sulfate and reactive sulfide reservoirs as sulfur is microbially cycled between them. A fundamental assumption in the model is that any reoxidation flux from reactive sulfide to seawater sulfate ultimately occurs through disproportionation reactions.

A series of model calculations were run incorporating the whole range in $^{33}S/^{32}S$ and $^{34}S/^{32}S$ fractionations observed in pure and enriched culture experiments (*21*). Inputs to the model are (i) the experimentally calibrated $^{33}S/^{32}S$ and $^{34}S/^{32}S$ fractionations associated with SRP and SDP, (ii) the isotopic composition of the sulfate entering the model through the seawater sulfate reservoir (the origin in Figs. 1 to 3), (iii) the proportion of sulfate entering the model through the seawater sulfate reservoir that leaves the model as pyrite rather than as sulfate minerals (f_{py}), and (iv) the proportion of sulfur entering the reactive sulfide pool that is completely reoxidized to sulfate ($f_{r\text{-}o}$). We began each calculation by choosing fractionations for SRP and SDP. By varying f_{py} and $f_{r\text{-}o}$, a unique array of relationships between the $\delta^{34}S$ and $\Delta^{33}S$ of model seawater sulfate $\{\Delta^{33}S = \delta^{33}S - [(\delta^{34}S/1000+1)^{0.515} - 1] \times 1000\}$ (*22*, *23*) was produced (fig. S2).

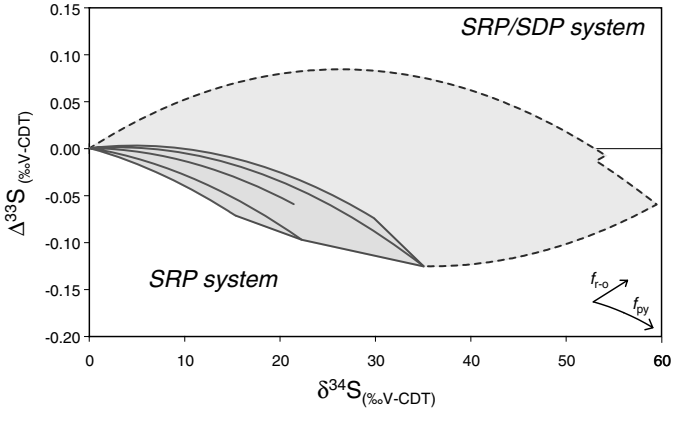

Fig. 1. $\Delta^{33}S$ versus $\delta^{34}S$ values for seawater sulfate predicted from an open-system steady-state S cycle model. The discrete curves are calculated for a sulfur cycle that includes only SRP. Different curves are calculated for different values of experimentally constrained isotopic fractionations by SRP (table S2). The field bound by a solid line is accessible to a strict SRP S cycle (SRP system). The field bound by a dashed line is accessible to a combined SRP-SDP microbial S cycle (SRP/SDP system). (**Inset**) The direction that $\Delta^{33}S$ versus $\delta^{34}S$ trajectories evolve as f_{py} and $f_{r\text{-}o}$ increase. The different $\Delta^{33}S$ versus $\delta^{34}S$ regions accessed by the SRP and SRP/SDP systems are used to assess the microbial contribution to the oceanic sulfur cycle at the time of sulfate deposition. Modeled and measured isotopic compositions are standardized to the V-CDT (Vienna Canyon Diablo Troilite) scale.

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As f_{py} increases in a model system including only SRP, the composition of seawater sulfate becomes ^{34}S enriched relative to the sulfate entering the model (Fig. 1) (*5*, *11*, *24*–*26*). In this case, the $\Delta^{33}S$ values of model seawater sulfate become more negative as $\delta^{34}S$ values increase (*18*). This is reflected in the orientation of the curves that outline the field of $\Delta^{33}S$ and $\delta^{34}S$ in Fig. 1 labeled SRP system. When the model S cycle is expanded with a reoxidative subcycle that allows for microbial sulfur disproportionation, increasing $f_{r\text{-}o}$ leads to seawater sulfate that is more enriched in ^{34}S and has more positive $\Delta^{33}S$ than when only SRP are included. This is reflected by the field labeled SRP/SDP system (Fig. 1). These model results form the basis for the use of the isotopic composition of proxies for seawater sulfate to distinguish the role of microbial sulfur disproportionation within the global sulfur cycle.

We measured the sulfur isotopic composition of 49 Proterozoic to Cambrian sulfate samples from either carbonate-associated sulfate (CAS) (35 in total) or sulfate minerals (14 in total) (table S1). In Fig. 2, the $\Delta^{33}S$ and $\delta^{34}S$ values of these samples are plotted relative to fields for the modeled SRP system and the modeled SRP/SDP system (*27*). Our model interpretation of these measurements assumes that they represent a well-mixed, homogeneous seawater sulfate reservoir whose composition is set by global processes. The majority of the Neoproterozoic/Cambrian data in Fig. 2A occupies the modeled SRP/SDP field. This $\Delta^{33}S$ and $\delta^{34}S$ evidence for active microbial sulfur disproportionation is consistent with phylogenetic studies and previous interpretations of the $\delta^{34}S$ record (*1*). Our approach, however, also yields evidence for an active SRP/SDP system in the Mesoproterozoic (Fig. 2B), leading to the suggestion that microbial sulfur disproportionation was not initiated in the Neoproterozoic but instead operated for at least part of the Mesoproterozoic (*17*).

The isotopic composition of seawater sulfate from the Mesoproterozoic Society Cliffs Formation [~1200 million years (My) old] and the Dismal Lakes Group (~1300 My old) shows evidence for active microbial sulfur dispro-

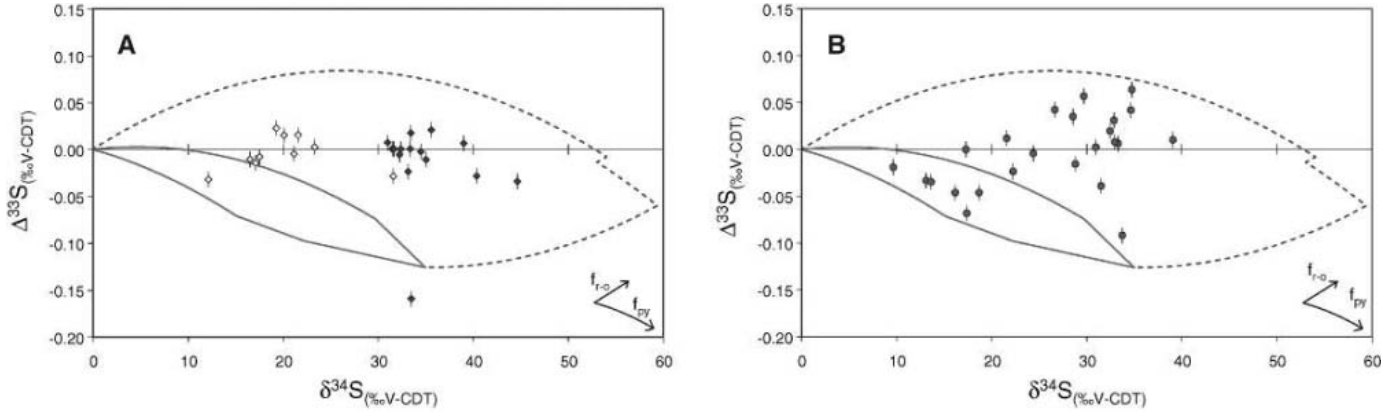

Fig. 2. Measured $\Delta^{33}S$ versus $\delta^{34}S$ values for Proterozoic-Cambrian seawater sulfate proxies combined with model predictions (Fig. 1). Measurement uncertainties are 0.008‰ in $\Delta^{33}S$ (shown in figure) and 0.12‰ in $\delta^{34}S$ (smaller than symbol size) for all data reported. (**A**) Neoproterozoic-Cambrian data (1000 to 500 Ma) divided into older (1000 to 750 Ma; open diamonds) and younger (571 to 500 Ma; solid diamonds) groups. Variation between the two groups likely reflects differences in f_{py} in a system with both SRP and SDP. (**B**) Paleo- to Mesoproterozoic data (2000 to 1000 Ma; solid circles). The Paleo- and Mesoproterozoic data extend across a range that is defined by the SRP system and the SRP/SDP system.

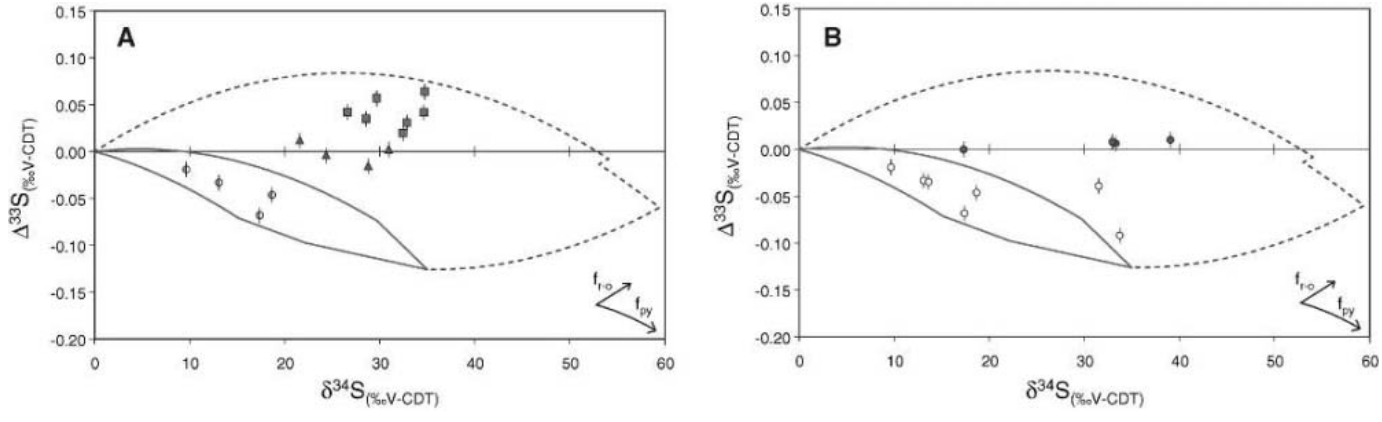

Fig. 3. Measured $\Delta^{33}S$ versus $\delta^{34}S$ values for seawater sulfate proxies from Mesoproterozoic basins and model predictions (Fig. 1). (**A**) The ~1200-My-old Society Cliffs Formation (solid squares) and the ~1300-My-old Dismal Lakes Group (solid triangles) require active sulfur disproportionation at the time of their deposition. Samples from the ~1450-My-old Helena Formation (circles) fit within the bounds of a strict SRP system and do not require the presence of SDP. (**B**) Measured $\Delta^{33}S$ versus $\delta^{34}S$ values from pre-1300-My-old basins (circles) plot, for the most part, in the SRP system field. Solid circles for the ~1660-My-old McNamara Group span almost the complete range of values observed for Proterozoic sulfate and exhibit a linear correlation with $\Delta^{33}S$ measurements. These isotopic systematics are consistent with the exclusive operation of SRP on a limited sulfate pool.

portionation (Fig. 3A). The Society Cliffs data contain a strong SDP signature, and the relation between this data and the model indicates extensive sulfur processing through disproportionation reactions. The $\Delta^{33}S$-$\delta^{34}S$ data for Dismal Lakes samples also contain an SDP signature and are consistent with lower proportions of sulfide reoxidation and pyrite burial. In contrast, the isotopic compositions of CAS in the ~1450-My-old Helena Formation are consistent with a strict SRP system (Fig. 3A) and do not require the influence of SDP. Our data indicate that SDP became progressively more important in the global sulfur cycle over the ~250-million-year time interval from 1450 to 1200 My old. Although these conclusions should be confirmed with additional data from other Mesoproterozoic basins, most pre-1300-My-old samples in the current data set exhibit $\Delta^{33}S$-$\delta^{34}S$ values that unambiguously reflect an SRP-only system (table S1 and Fig. 3B).

Thus far, samples from only one pre-1300-My-old sedimentary basin (McNamara Group, ~1660 My old) (Fig. 3B) appear to be inconsistent with the conclusions drawn above. These data, however, display some unusual isotopic characteristics. The $\delta^{34}S$ values of these samples span a wide range (~17 to 39‰), covering a substantial portion of the entire data set (~9 to 44‰). In addition, McNamara $\Delta^{33}S$ values vary in a near-linear fashion with $\delta^{34}S$ values. Both of these characteristics are indicative of Rayleigh fractionation, and we can reproduce the McNamara data with such a model involving only SRP (*21*). Although we cannot rule out the possibility that the McNamara samples retain isotopic evidence of the effects of disproportionation, we hypothesize that this formation records a sulfur cycle dominated by SRP operating on a limited sulfate pool. This hypothesis is consistent with recent discussions of low sulfate concentrations during the deposition of the McNamara Basin sediments (*11*, *28*), and it is testable by sulfur isotope analysis of sedimentary sulfides that formed contemporaneously with carbonates of the McNamara Group (*29*).

Taken together, our results bracket the appearance of a globally significant disproportionation pathway between 1450 and 1300 Ma. This predates prior estimates by several hundred million years (*1*) and exposes an inherent limitation of the use of $\delta^{34}S$ to explore biogeochemical aspects of the sulfur cycle. Positive $\delta^{34}S$ evidence for SDP requires that the fractionations expressed in the isotope record must exceed the extreme fractionations observed for SRP (*1*). By contrast, ^{33}S traces the contribution of microbial disproportionation at smaller $^{34}S/^{32}S$ fractionations that would seem to be completely consistent with sulfate reduction from $\delta^{34}S$ alone.

Although the new ^{33}S measurements suggest a major change in the microbial regimes that controlled the isotopic composition of Proterozoic seawater sulfate, the environmental impetus for this change is less clear. The intermediate sulfur compounds required for SDP are generated by chemical oxidation of sulfide by O_2 and metal oxides (*1*, *14*), by photosynthetic sulfide oxidizers (*1*), and by O_2- or nitrate-respiring anaerobic nonphotosynthetic sulfide oxidizers (*1*, *14*). On modern Earth, the compounds produced by these processes occur in a variety of chemical transition zones, such as at oxic-anoxic interfaces in marine sediments and stratified water columns and within the layers of microbial mat communities (*15*). We suggest that sulfur disproportionation dominantly occupied surface ocean and/or shelf environments where local oxidative processes were responsible for the production of sulfur intermediates. Other indicators of an oxidative surface environment, such as $\delta^{13}C$ variations (*11*, *30*), evolutionary arguments (*1*, *31*), and sulfate concentration estimates (*11*, *28*), are temporally consistent with a Mesoproterozoic onset of disproportionation. A high-resolution ^{33}S record from the critical interval between 1450 and 1300 Ma may capture this onset in action, revealing whether the rise of SDP lagged or accompanied the progressive oxygenation of Earth's surface.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5753/1477/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 and S2

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Electrowetting in Carbon Nanotubes

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We demonstrate reversible wetting and filling of open single-wall carbon nanotubes with mercury by means of electrocapillary pressure originating from the application of a potential across an individual nanotube in contact with a mercury drop. Wetting improves the conductance in both metallic and semiconducting nanotube probes by decreasing contact resistance and forming a mercury nanowire inside the nanotube. Molecular dynamics simulations corroborate the electrocapillarity-driven filling process and provide estimates for the imbibition speed and electrocapillary pressure.

The long inner hollow core of carbon nanotubes offers opportunities for the study of fluid flow in nanoconduits (*1*), chemical reactions in nanosize test tubes (*2*), and electronic and magnetic behavior in encapsulated nanowires (*3*, *4*). Several methods have been devised to fill nanotubes with metals (*3*–*5*) and other materials (*6*–*11*), resulting mostly in the formation of discontinuous nanowires.

Capillarity has been shown to drive wetting and filling of carbon nanotubes with liquids, provided that the surface tension of the liquid is less than ~180 mN/m (*10*, *11*). Most pure metals, which possess surface tension greater than 200 mN/m, cannot be incorporated spontaneously into nanotubes, thus hindering the fabrication of metal nanowires by this simple method (*7*, *9*). Because liquid mercury was shown not to wet carbon nanotubes (*10*), immersion into mercury has served to provide a good Ohmic contact for measuring the electrical properties of individual nanotubes attached to scanning probe tips (*12*–*14*). Such measurements, however, can be affected by the application of an electrical potential across the nanotube-mercury interface, which can lower the surface tension and cause electrowetting (*15*). We now show that electrically activated wetting of carbon nanotubes is indeed possible, with important implications for nanofluidics, nanoprobes, nanowires, and controllable manipulation at the nanoscale.

Single-wall carbon nanotubes (SWNTs) were grown by chemical vapor deposition on a silicon wafer decorated with iron nanoparticles (*16*). We attached these SWNTs to gold-coated silicon atomic force microscope (AFM) tips to form "nanotube probes" using the pickup technique of Hafner *et al*. (*17*). Picked-up SWNTs with suspended length between 200 to 600 nm were selected for further processing. The tubes had an average diameter (±SD) of 5 ± 1 nm, larger than those (1 to 3 nm) reported by Hafner *et al*. (*17*) and were defect-free, as inferred from transmission electron microscopy (TEM) images.

After annealing for 36 hours at 180°C, each nanotube probe was subjected to electric pulse etching (1.5 to 3.5 V for 20 μs) against a fresh highly oriented pyrolytic graphite surface, as described elsewhere (*16*, *17*). The etching shortened the SWNT (to between 50 and 200 nm), which reduced bending and buckling effects and opened up its suspended free end to facilitate access of its inner core by wetting.

In ambient conditions, the shortened SWNT probe was brought into contact with a fresh droplet of liquid mercury (diameter of ~200 μm) by engaging the probe in tapping mode on the droplet surface with a Digital Instruments AFM and a Nanoscope IV controller. Both the total length of the SWNT and the length immersed in the mercury drop could be determined (*18*). Precautions were taken to prevent the gold-coated silicon tip from contacting the mercury surface directly because mercury dissolves the gold, causing nanotube loss. After the SWNT was immersed into Hg by 17 ± 2 nm, electrical dc potentials were applied to the nanotube probe while tip conductance was monitored (with the Hg at ground). Resistances were measured at low bias (100 mV). Two types of experiments were performed. For a fixed-probe position, current-voltage (*I-V*) curves were recorded. Alternatively, the SWNT was lifted from the mercury surface at fixed applied potential in order to measure the pull-off force acting on the tip.

Typical *I-V* curves for shortened nanotube probes are shown in Fig. 1. Initially, low currents (2 to 10 μA) were measured for potentials between +1 to –1 V (curve I to II). The probe of Fig. 1A has a low bias resistance of 208 ± 20 kilohms, consistent with the range of values reported for good contact between metallic SWNTs and gold-coated AFM tips (*13*). The probe of Fig. 1B has a resistance of 1.65 ± 0.29 megohms and a slightly asymmetric *I-V* curve (I to II), both indicative of a semiconducting SWNT. Probe resistances from a large number of metallic SWNTs ranged from 100 to 300 kilohms, with no apparent correlation with the length of the truncated SWNT (table S1). Semiconducting SWNTs had substantially higher resistances (1 to 3 megohms). The *I-V* curves were stable with voltage cycling between –1 and +1 V.

Increasing the voltage to a threshold value between ±1 and ±2 V while keeping the nanotubes immersed in the mercury at fixed depth led to an abrupt and large increase in conductivity (curve II to III), for both the metallic and semiconducting SWNTs. The jump to high current (termed "probe activation") occurred at –1.15 V for the metallic SWNT probe (Fig. 1A). Further increase in the magnitude of the applied voltage to –1.50 V caused a slight variation in current. Subsequent cycling of the voltage between ±1.5 V results in stable *I-V* curves at elevated currents (curves III to IV) for 10 to 15 cycles. The low bias resistance of the metallic SWNT probe in its activated state decreased to 29 ± 4 kilohms. The semiconducting SWNT probe exhibited a similar response (Fig. 1B); probe activation occurred at –1.26 V and its resistance dropped to 46.8 ± 2.9 kilohms, notably near the value for the activated metallic SWNT in Fig. 1A.

In addition to negative potentials, probe activation also occurs consistently at similar (absolute) positive potentials (fig. S1). For

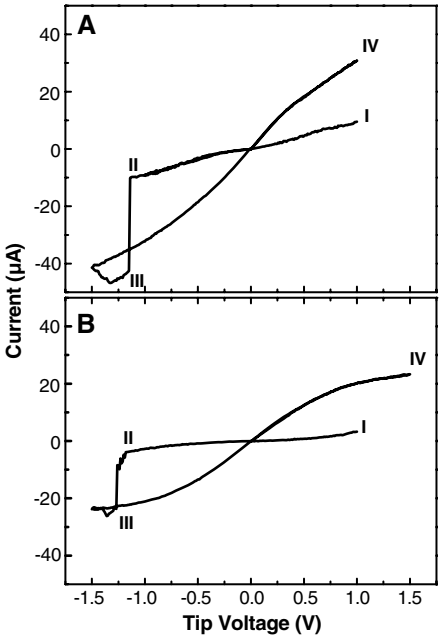

Fig. 1. Probe current as a function of applied tip voltage for (**A**) an 80-nm-long metallic SWNT and (**B**) a 130-nm-long semiconducting SWNT. Both nanotubes are immersed by 17 ± 2 nm into a mercury droplet. Curve I to II corresponds to the low-conductivity state. Curve II to III indicates the abrupt transition to the high-conductivity state, which is described by curve III to IV.

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negative voltage sweeps, average activation thresholds were –1.5 ± 0.5 V for metallic and –1.3 ± 0.3 V for semiconducting SWNTs. After activation, and so long as the SWNT tip remained immersed, the high-conductivity behavior was maintained upon voltage cycling through zero bias, although a drift to higher resistances was seen at longer times (>5 min). When the SWNTs were completely removed from the mercury surface, they reverted to a low-conductivity state (*19*), although the corresponding resistance was generally lower than that before the first round of activation (table S1). The probe could be reactivated at a lower voltage than the initial threshold established previously; the high-conductivity state was then fully recovered.

Simultaneous measurement of the tip pull-off force from numerous force-distance curves with tip voltage indicated that probe activation coincides with a roughly fivefold increase in the attraction between the SWNT probes and mercury (Fig. 2A). This trend was consistent and readily observable for both metallic and semiconducting SWNTs (Fig. 2B). Relatively weak pull-off forces between 1 to 5 nN were measured before activation, as compared with strong forces of 11 to 30 nN after activation.

We attribute these observations to electrically activated wetting and filling of our SWNTs by mercury. Electrowetting has been demonstrated in microchannels (*15*) but not at the nanoscale. Also known as electrocapillarity, the effect is based on electrostatic control of the solid-fluid interfacial tension (*20*). After the application of a potential, charge builds up across the SWNT-mercury contact (*21*). The repulsion between similar electric charges present at the mercury surface lowers the surface tension (*22*). At a critical value of the applied potential, wetting may commence that forces mercury up along the SWNT sidewalls and also fills the SWNT core to form a continuous metallic nanowire. Mercury transported in this manner to the AFM tip can react with the gold to form an amalgam, which will alter the upper contact characteristics. Pumping enough mercury along the SWNT walls or through its core may even liquefy the upper contact and detach the SWNT from the tip.

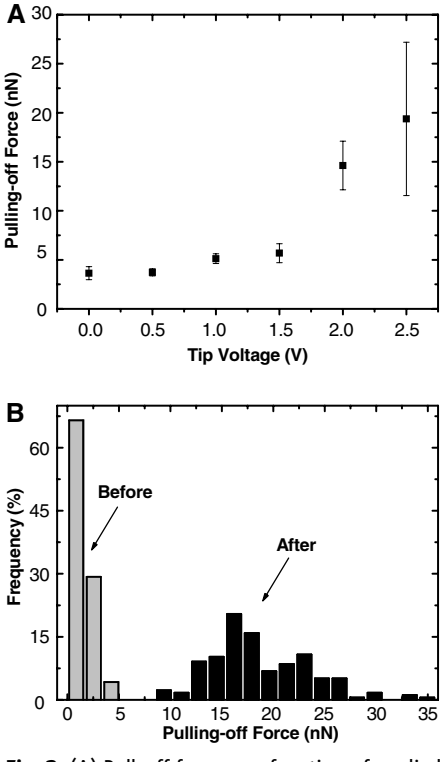

Fig. 2. (**A**) Pull-off force as a function of applied tip voltage for a 120-nm-long semiconducting carbon nanotube. The force is measured by extracting the nanotube from the mercury surface at the corresponding voltage. Error bars show means ± SD. (**B**) A histogram of pull-off forces measured by using 14 different SWNT probes. The "before-activation" region corresponds to 188 force-distance curves from six nanotubes recorded at a tip bias of 0 V. The "after-activation" region corresponds to 176 force-distance curves from eight nanotubes, recorded at a tip bias of ±2 V.

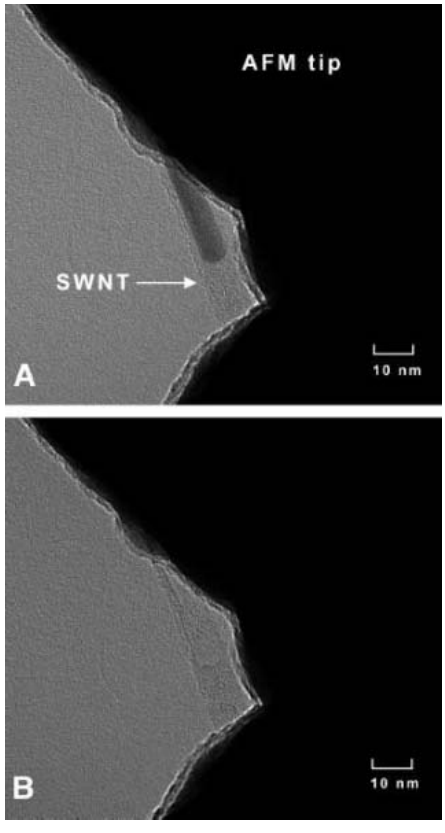

Fig. 3. (**A**) Transmission electron micrograph of a segment of an activated 150-nm-long SWNT attached on a gold-coated AFM tip. Material of darker contrast fills the upper core of the nanotube, forming a highly curved meniscus with a contact angle of 150° ± 5°. (**B**) The same region after focusing the TEM electron beam on the material inside the nanotube. A faint trace is left behind at the position of the meniscus.

These hypotheses led us to look for evidence of mercury trapped inside the activated SWNTs and/or gold coating loss from the AFM tip. We examined more than 50 activated SWNTs by ex situ TEM for evidence that mercury had penetrated and filled the inner core (*23*). Finding such evidence was difficult because extraction of the SWNT tip from the mercury surface effectively terminates electrowetting and allows mercury to evaporate. Nevertheless, we found two instances with clear evidence of material trapped inside the SWNT. The first case pertains to a 150-nm-long SWNT, a segment of which is visible in Fig. 3A along the sidewall of the AFM tip. Darker material inside the SWNT formed a highly curved meniscus with a contact angle of 150° ± 5°, notably close to that of mercury on graphite (*24*). Focusing the TEM electron beam on this material caused it to vanish but left behind a faint trace of the curved interface (Fig. 3B). We hypothesize that the material inside was liquid mercury in its nonwetting state and that heating from the electron beam made it move or evaporate rapidly, rendering its identification impossible.

The second case of TEM evidence for material inside an activated SWNT is presented in Fig. 4A. The contrast is sufficient to discern a darker material in the center of the lower half of the SWNT. Readily apparent in Fig. 4B is the dissolution of the gold-coating at the AFM tip apex, which exposes the silicon tip. Although this tip did not touch the mercury surface, its appearance is identical to that of a coated tip without a SWNT (Fig. 4C) that had been briefly immersed into mercury deliberately. Mercury must have been transported to the tip by some other mechanism. We considered four possibilities: evaporation, thermomigration, electromigration, or electrowetting. Mercury evaporation at room temperature requires long times (>15 hours) to dissolve 40-nm-thick gold films (*25*) and must be excluded. In the absence of wetting, thermomigration (*26*) would compete against surface tension (*27*). Electromigration is polarity dependent (*28*), whereas the phenomenon we describe here is not. Thus, the only plausible mechanism for mercury transport from the drop to the AFM tip is electrowetting.

We have performed molecular dynamics simulations (*18*) of SWNTs interacting with a mercury bath to help visualize the wetting process. Lippmann's model of electrowetting (*20*) was adopted for a cylindrical capacitor, defined between the nanotube and a column of mercury, with a potential V applied across the plates (*21*). When a neutral Hg atom is brought to the interface from the bulk of the liquid, the surface charge is redistributed over a greater area because of electrostatic repulsion. The free energy of the interface is then lowered by

an amount equal to the change ΔE_e in electrostatic energy:

$$\Delta E_e = -cV^2/2\rho_s \quad (1)$$

where $c = C/A$ is the capacitance per unit area and $\rho_s = 11.82\ \mathrm{nm}^{-2}$ is the surface density of mercury. The energy drop was realized through a local external force acting along the surface normal on each atom near the interface according to:

$$F_{ext}(r) = \begin{cases} F_0, & 0 < r < a \\ 0, & r \geq a, r \leq 0 \end{cases} \quad (2)$$

where r is the normal distance to the surface, $a = 3.5$ Å is a characteristic distance, and $F_0 = \Delta E_e/a$. The direction of the force is toward the surface, and a is chosen to include atoms from the second surface layer. Although a simplification, this model contains the salient features of electrowetting and should give a reasonable description of its dynamics.

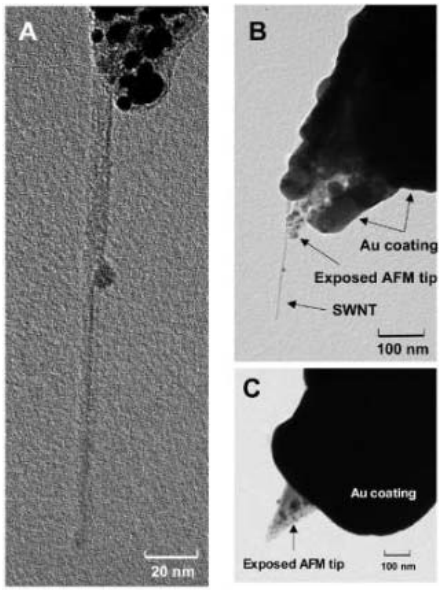

Fig. 4. (**A**) Transmission electron micrograph of an activated 180-nm-long SWNT attached to a gold-coated AFM tip. Material of darker contrast fills the lower half portion of the nanotube core. The material appears to terminate near a bulge or particle on the outside of the nanotube. The position of the bulge coincides with the outer edge of the coating before removal. Beyond the bulge, the nanotube diameter appears to be larger, suggesting the presence of a second nanotube terminating at the bulge. (**B**) Zoom-out of micrograph (A), indicating that the gold-coating at the AFM tip apex has been dissolved by mercury (leaving separated domains of AuHg amalgam on the Si tip), although no direct contact with the mercury surface has been made. (**C**) Micrograph of a different gold-coated AFM tip without a nanotube, which has been dipped into mercury deliberately to expose the Si tip apex by gold dissolution.

Simulating long (40,40) SWNTs, near the size of the experimental ones, with correspondingly large mercury baths was computationally prohibitive. Instead, we simulated (*29*) the immersion of a 15.7-nm-long uncapped (20,20) SWNT in a configuration similar to that suggested by Supple and Quirke (*30*). In the equilibrium state of an immersed SWNT without applied voltage, a nonwetting meniscus forms on the outside and mercury does not penetrate the open unblocked end (Fig. 5A). For the same nanotube 1.5 ns after the application of 3.5 V, which is larger than the calculated threshold of 2.5 V for electrowetting (*31*), mercury has filled the core and wetted the outside walls (Fig. 5B). Indeed, wetting begins immediately after the potential is turned on and the liquid moves inside as a single front at a speed of ~13 m/s, whereas a thin film spreads on the outer wall. Of course, a closed SWNT capped at the free end would only be wetted at the outside (fig. S4).

The mass-transport rate of Hg atoms through the SWNT core depended quadratically on the applied potential (fig. S5), a scaling that deviates from the linear dependence predicted by the Lucas-Washburn equation for filling of macroscopic capillaries (*30*, *32*). At an applied voltage of 3.5 V, the imbibition speed of mercury is 2.7×10^{12} atoms per second. An estimate (*33*) of the amount of Au (1.55×10^8 atoms) dissolved from the AFM tip of Fig. 4B suggests that the Hg transport rate is more than sufficient to account for the removed Au. The electrocapillary pressure (ECP) was also calculated to have a quadratic dependence on applied potential (fig. S6), in agreement with observations in microchannels (*15*). Typical ECP calculated values are in the range of 0 to 3 kbar (for potentials between 2.5 and 4.5 V), indicating a large driving force for wetting.

The simulations offer a starting point for explaining experiments. Electrowetting is an activated process with a threshold voltage. Once that voltage is exceeded, mercury imbibes into the SWNT core and is also transported along the outer sidewalls, rapidly reaching the Au-coated AFM tip, where it reacts with gold to form an amalgam. As more Hg arrives, the upper contact is liquefied and becomes more similar to the lower SWNT-Hg contact, that is, more Ohmic (*13*). The voltage drop across the upper contact decreases, which increases the applied potential across the lower contact. This high potential, in turn, increases the ECP and the Hg transport rate to the AFM tip. Thus, the observed improvement in conductance is a result of better wetting at the lower contact, changes in the upper contact in response to the supply of Hg, and the formation of a column of Hg in the SWNT core.

Experiments with SWNTs that have not undergone pulsed etching and are believed to be closed consistently exhibit larger electrowetting thresholds and never reach the high levels of conduction seen with open SWNTs. This observation suggests that the Hg column that forms in the SWNT core must contribute to the lowering of the probe resistance. Indeed, in one case of a 270-nm-long individual SWNT, we measured an activated probe resistance of 3.79 ± 0.18 kilohms (table S1), equivalent to four times the conductance quantum $G_0 = (12.9\ \text{kilohms})^{-1}$. In addition, the metal-filled core may be responsible for the marked increase in conductance of initially semiconducting SWNTs upon probe activation.

When nanotubes are removed from the mercury surface, electrowetting ceases, and the inner Hg column rapidly disintegrates by draining (*34*) and evaporation because wetting is no longer supported. Once Hg is no longer supplied to the upper contact, the remaining Hg diffuses into the surrounding Au to form a solid AuHg amalgam. This amalgam at the upper contact has a lower work function than pure Au (*35*), resulting in probe activation at

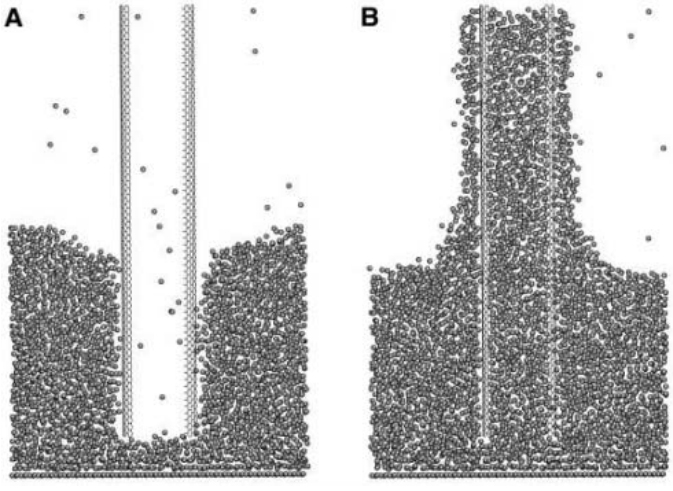

Fig. 5. Results from molecular dynamics simulations of a 15.7-nm-long (20,20) SWNT immersed into a mercury bath at 300 K (**A**) before (at time zero with no applied bias) and (**B**) after probe activation (1.5 ns after application of a potential of 3.5 V).

a reduced threshold voltage upon reimmersion. Electrowetting is by definition an attractive interaction between mercury and SWNT and, thus, the force required to pull a SWNT off a mercury surface should be larger in an activated state than that of a nonactivated state (*36*).

Electrowetting in carbon nanotubes may offer opportunities for studies of nanofluidic transport. It can also be exploited for the formation of continuous nanowires crystallized in one dimension from low–melting point metals (e.g., Ga and In), enabling the measurement of the intrinsic electrical and magnetic properties of encapsulated nanowires. Such structures, attached to AFM tips, could serve as robust nanoelectrode probes with increased current load capacity and enhanced imaging capabilities.

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32. The quadratic scaling can be predicted through the Lucas-Washburn equation by introducing a velocity-dependent dynamic contact angle or, correspondingly, a "wetting-line friction" as proposed by Martic *et al*. (*38*).
33. To estimate the amount of the dissolved Au, we assume a pyramidal AFM tip with conformal Au coating, which terminates into a spherical apex. We also assume that 30% of the Au atoms in the remaining Au coating with the lighter contrast have formed an amalgam.
34. The simulation predicts that mercury will rapidly drain from the (20,20) SWNT core at a speed of 15 m/s when the applied potential is decreased to zero.
35. The work function of Au (5.1 eV) is greater than that of Hg (4.6 eV) and the amalgam value should be in between (*39*).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5753/1480/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References

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A Well-Preserved *Archaeopteryx* Specimen with Theropod Features

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A nearly complete skeleton of *Archaeopteryx* with excellent bone preservation shows that the osteology of the urvogel is similar to that of nonavian theropod dinosaurs. The new specimen confirms the presence of a hyperextendible second toe as in dromaeosaurs and troodontids. *Archaeopteryx* had a plesiomorphic tetraradiate palatine bone and no fully reversed first toe. These observations provide further evidence for the theropod ancestry of birds. In addition, the presence of a hyperextendible second toe blurs the distinction of archaeopterygids from basal deinonychosaurs (troodontids and dromaeosaurs) and challenges the monophyly of Aves.

The Archaeopterygidae from the Late Jurassic of Germany are recognized as the earliest undisputed fossil avians (*1*, *2*). Archaeopterygids have been known from nine skeletal specimens (*3*, *4*), and most of these are fragmentary or poorly preserved. As a result, crucial features of their osteology have remained uncertain or entirely unknown (*3*, *5*).

Here we describe a 10th skeletal specimen of an archaeopterygid (*3*, *4*). The specimen was discovered in an unknown locality of the Solnhofen area and was housed in a private collection before it was recently acquired by the Wyoming Dinosaur Center, Thermopolis, USA (collection number WDC-CSG-100; a cast will be deposited in Forschungsinstitut Senckenberg).

The "Thermopolis specimen" is a slightly dissociated skeleton on a single slab of pure limestone. Wing and tail feather impressions are well preserved (Fig. 1). In size and osteology, the new specimen corresponds best with the Munich specimen, which is the holotype of *Archaeopteryx bavarica* (*6*) (table S1). However, because there is an ongoing controversy about the taxonomic composition of the Archaeopterygidae, which currently include two genera, *Archaeopteryx* and *Wellnhoferia* (*3*, *7*), we do not assign the new specimen to a particular species in the present study.

The skull is the best-preserved one of all archaeopterygids and the only one that is exposed in dorsal view. There are two accessory antorbital openings, which were recognized in the Eichstätt specimen but whose presence was recently questioned (*3*, *8*). They are part of the maxillary bone (*9*, *10*) and not

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of the mesethmoid (*2*), and thus are homologous to the maxillary and promaxillary fenestrae of theropod dinosaurs (*11*, *12*) (Fig. 2).

The dorsal surface of the midsection of the right palatine bone is visible through the antorbital fenestra (Fig. 2). Apart from minor differences in proportions, this bone closely resembles the isolated palatine of the holotype of the Munich specimen (*13*). However, in contrast to the latter, it also exhibits a short jugal process (Fig. 2) and is thus tetraradiate as in nonavian theropods and not triradiate as in ornithurine birds. Because we do not think that there was such a great morphological discrepancy between the otherwise similar specimens, and because there appears to be a breakage line in the holotype of the Munich specimen, we assume that part of the lateral margin of the palatine of the Munich specimen is broken.

The ectopterygoid is preserved in its original position (*8*), and the hook-shaped jugal process contacts the jugal. The temporal region is difficult to interpret and apparently not completely preserved, because neither a squamosal nor a quadratojugale (*13*) can be discerned.

Nearly the entire right coracoid is visible in cranial view, a bone whose shape in *Archaeopteryx* has been uncertain and of which remarkably different reconstructions exist (*2*, *14*, *15*). The body is of subrectangular shape and bent craniocaudally, with a concave lateral margin and a well-developed lateral process (Fig. 3). In its shape it resembles the

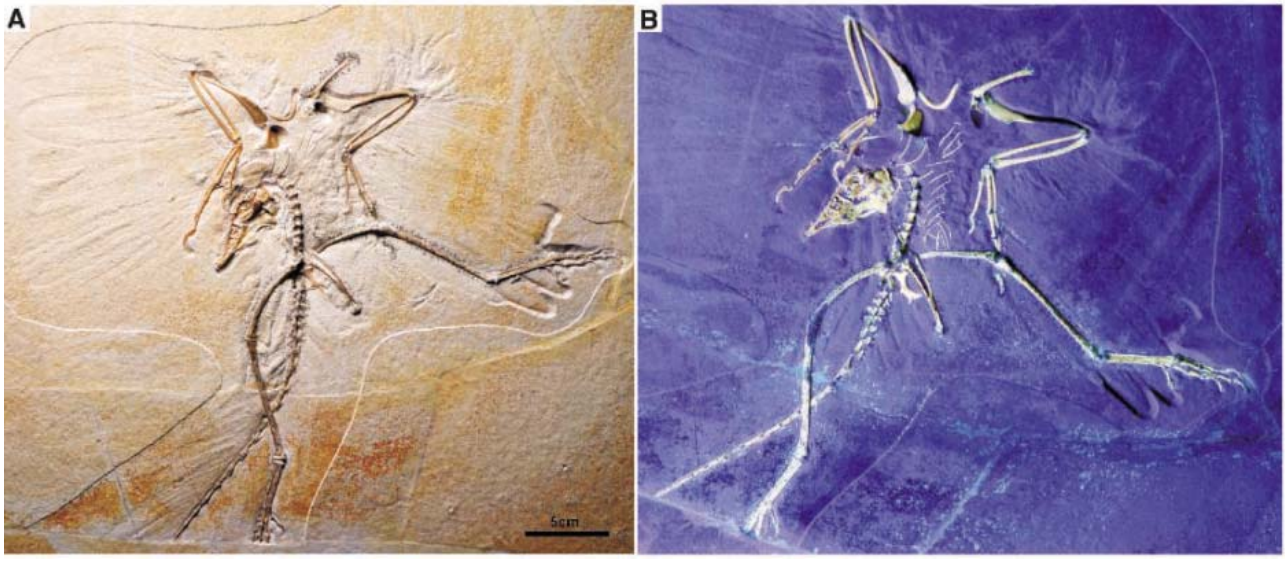

Fig. 1. The 10th skeletal specimen of the Archaeopterygidae (collection number WDC-CSG-100) in ventral view. (**A**) Skeleton with wing and tail feather impressions. (**B**) Ultraviolet-induced fluorescence photograph to show the preserved bone substance.

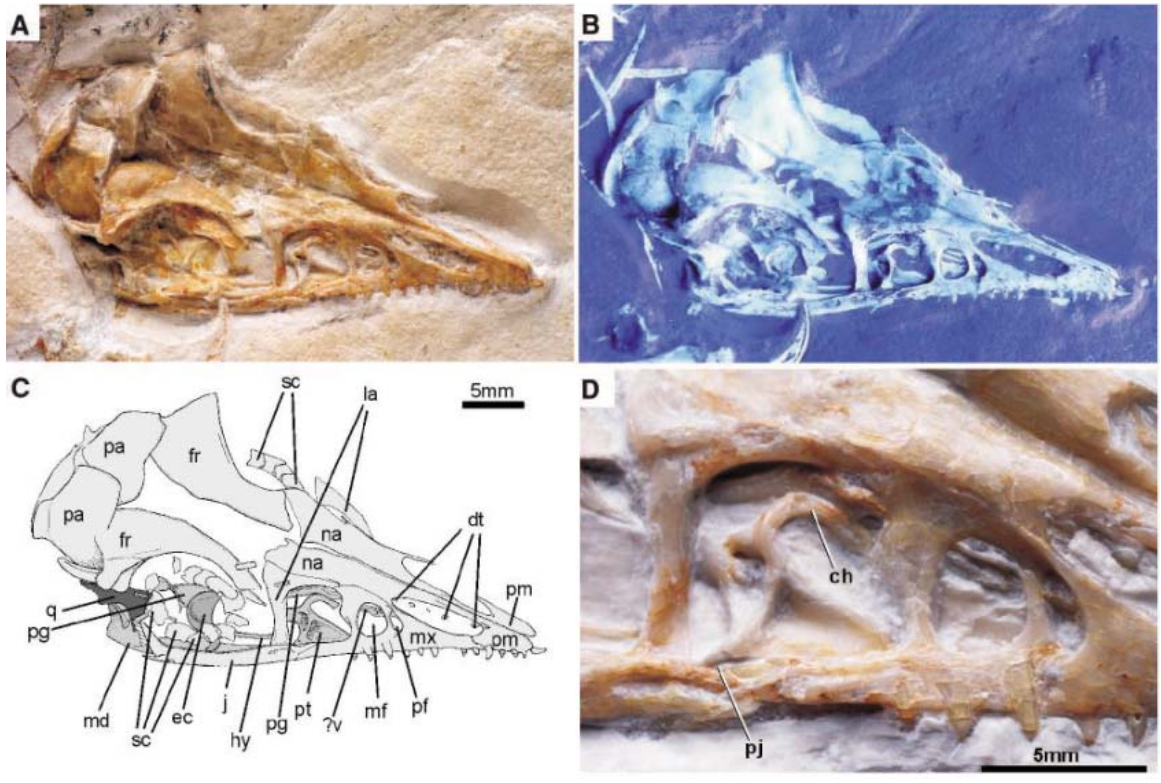

Fig. 2. Skull of the new *Archaeopteryx* specimen. (**A**) Overall view as preserved. (**B**) Ultraviolet-induced fluorescence photograph. (**C**) Interpretative drawing. ch, choanal process of palatine; dt, dentary teeth; ec, ectopterygoid; fr, frontal; hy, hyoid; j, jugal; la, lacrimal; md, mandible; mf, maxillary fenestra; mx, maxilla; na, nasal; pa, parietal; pf, promaxillary fenestra; pg, pterygoid; pj, jugal process of palatine; pm, praemaxilla; pt, palatine; q, quadrate; sc, plates of sclerotic ring; ?v, ?vomer. (**D**) Detail of antorbital fenestra with palatine bone.

coracoid of dromaeosaurs (*1*), although the biceps tubercle is more strongly developed. Coracoid and scapula are not fused, and in concordance with other specimens of the Archaeopterygidae (*14*), there are no ossified sternal plates.

The structure of the proximal tarsal bones of *Archaeopteryx* has played a role in discussions about the theropod ancestry of birds, with the controversy being about whether *Archaeopteryx* had an ascending process of the astragalus as in nonavian theropod dinosaurs (*1*, *16*) or whether there was a narrow "pretibial bone" as in neognathous birds (*2*, *17*–*19*). The new specimen shows the undistorted cranial surface of the tarsus. It is clearly visible that the astragalus forms a broad ascending process identical to that of theropod dinosaurs (*1*) (Fig. 3).

Contrary to virtually all existing reconstructions of *Archaeopteryx*, the new specimen shows that the first toe was not fully reversed as in extant birds. On both feet, the first metatarsal attaches to the medial surface of the second metatarsal as in theropod dinosaurs, not to its plantar surface as in extant birds with a retroverted first toe (*20*). The shaft of the first metatarsal does not exhibit the torsion that is characteristic of birds with a fully retroverted first toe (*20*). The proximal phalanx of the first toe further exposes its mediodorsal surface (Fig. 3). Because the metatarsals are visible in dorsal view, the dorsal aspect of this phalanx would not be visible if the first toe were fully reversed. All pedal phalanges are firmly articulated, and postmortal dislocation is unlikely to affect both feet in the same way. We thus conclude that the first toe of *Archaeopteryx* was spread medially and not permanently reversed as in extant birds. It has hitherto been unrecognized that the first toe exhibits the same position in the only preserved foot of the holotype of the recently established archaeopterygid taxon *Wellnhoferia* (*21*, *22*). In this specimen, the metatarsals are seen from their plantar side and the proximal phalanx of the first toe from its medioplantar side. The first toe of the left foot of the Berlin specimen also appears to have been spread medially rather than having been fully reversed. The feet of the London and Eichstätt specimens are preserved in lateral or medial view, and the impression of a reversed first toe in these specimens may thus be an artefact of preservation, because the medially spread toe is brought on a level with the sedimentation layer.

The absence of a fully reversed first toe indicates that *Archaeopteryx* did not have a perching foot and was at best facultatively arboreal (*3*).

In addition, the new specimen shows that *Archaeopteryx* had a hyperextendible second toe, as in Deinonychosauria (dromaeosaurs and troodontids) and the late Cretaceous bird *Rahonavis* (*12*, *23*). Although the proximal phalanx of the second toe of *Archaeopteryx* is not as abbreviated as in the latter three taxa (*3*), the ability to hyperextend this toe is clearly indicated by the proximodorsally expanded articular trochlea of its first phalanx (Fig. 3). The second toe bears a larger claw than the other digits, which is, however, not as greatly enlarged as in most (*24*) Deinonychosauria. This observation confirms the controversial presence of a dorsally expanded articular trochlea of the proximal phalanx of the second toe in the Eichstätt specimen (*3*, *11*, *24*). Whether its apparent absence in the London *Archaeopteryx* (*25*) is real or an artifact of preservation needs to be further examined; the dorsal part of the trochlea of the second toe of the other specimens is either not visible or is too poorly preserved for detailed examination.

Most workers consider Deinonychosauria to be the sister taxon of Aves (*26*–*28*), and the presence of a hyperextendible second toe in *Archaeopteryx* supports a close relationship between deinonychosaurs and avians. On the basis of current phylogenies (*26*–*28*), this feature must be regarded as an apomorphy of a clade (Deinonychosauria + Avialae) that is lost in birds that are closer to the extant species than are archaeopterygids and *Rahonavis*.

In order to evaluate how the data obtained from the new specimen affect the phylogenetic position of *Archaeopteryx*, we corrected char-

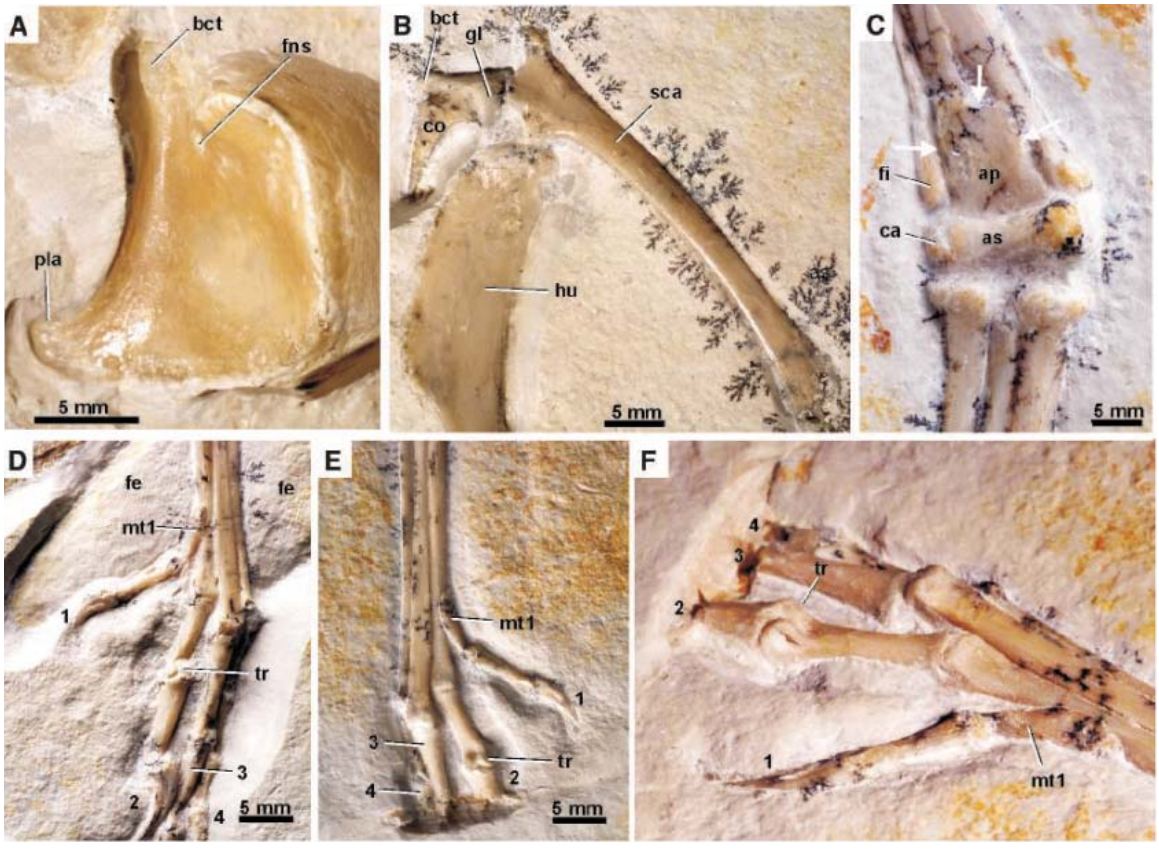

Fig. 3. Selected postcranial bones of the new *Archaeopteryx* specimen. (**A**) Right coracoid in cranial view. (**B**) Left coracoid in lateral view, proximal end of left humerus in caudal view, and left scapula in lateral view. (**C**) Right tarsus in cranial view. (**D**) Left foot in dorsal view. (**E** and **F**) Right foot in dorsal (E) and dorsomedial (F) view. as, astragalus; ap, ascending process of astragalus; bct, biceps tubercle; ca, calcaneus; co, coracoid; dt, dentary teeth; fe, feather impressions; fi, fibula; fns, foramen nervi supracoracoidei; gl, glenoid process of coracoid; hu, humerus; mt1, first metatarsal; pla, lateral process of coracoid; sca, scapula; tr, proximodorsally expanded articular trochlea of first phalanx of second toe. White arrows in (C) indicate the margins of the ascending process of the astragalus; pedal digits are numbered in (D) to (F).

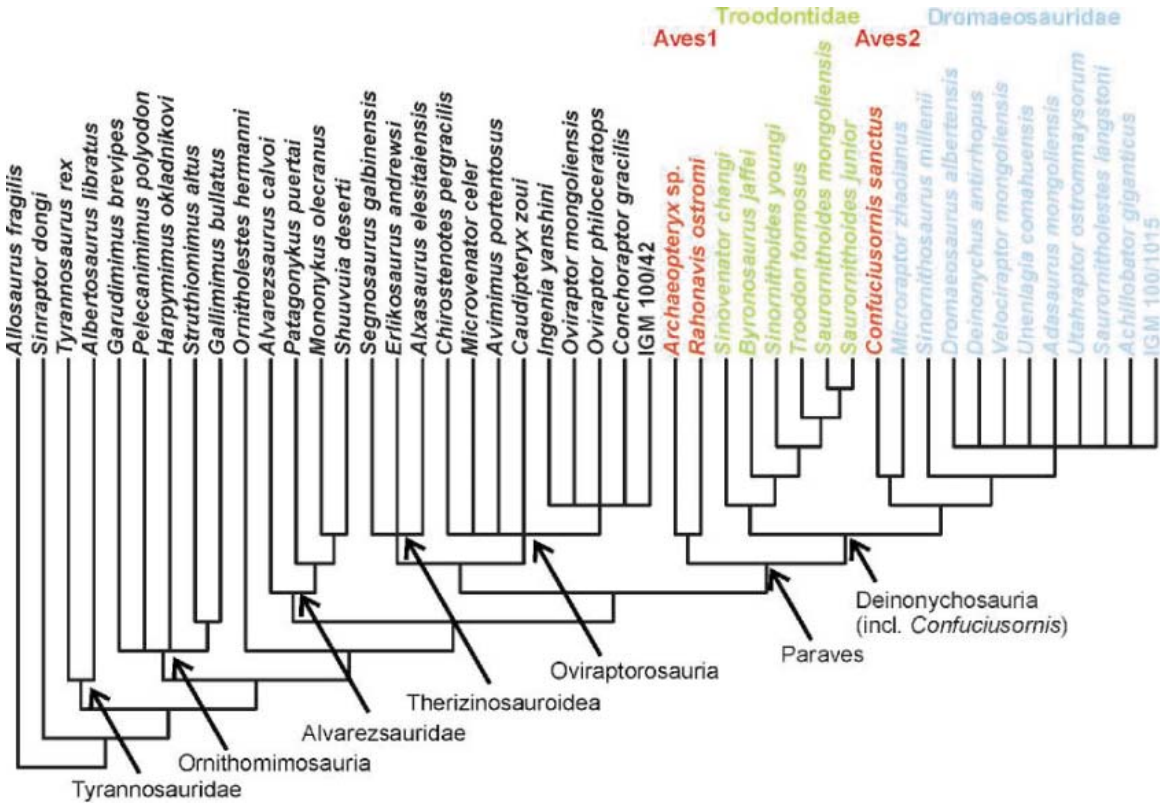

Fig. 4. Strict consensus tree of 288 most parsimonious trees (length, 599; consistency index, 0.42; retention index, 0.70) resulting from a phylogenetic analysis of the character matrix of (*26*) with NONA 2.0 (*29*). Eight characters were modified for *Archaeopteryx* as follows, according to our results and those in (*13*, *14*) [character (ch.) numbers refer to (*26*); character states and descriptions in brackets refer to the scoring used in this analysis): ch. 28:0 [quadratojugal L-shaped (*13*)], ch. 48:0 [palatine tetraradiate], ch. 106:– [not applicable, as there are no ossified sternal plates in the adult (*14*)], ch. 111:? [coracoid and scapula are unfused in most specimens, but fusion may have occurred in late ontogeny (*3*)], ch. 163:1 [ascending process of astragalus separated by transverse groove from condylar portion], ch. 166:0 [metatarsals not co-ossified], ch. 170:2 [newly added character state: penultimate phalanx of digit II modified for hyperextension but ungual not hypertrophied], ch. 171:0 [metatarsal I articulates to medial surface of metatarsal II]. One character was further modified for *Rahonavis*: ch. 111:0 [scapula and coracoid separate (*23*)]. Settings of the analysis and all other scorings are as in (*26*); *Allosaurus fragilis* and *Sinraptor dongi* were used as outgroup taxa. Character scoring for all taxa is given in the supporting online material.

acter scoring for this taxon in large character matrices that include birds and nonavian theropods (*26*) (Fig. 4). Reanalysis of the data did not support monophyly of the three included avian taxa but showed *Archaeopteryx* and *Rahonavis* to be outside a clade including *Confuciusornis* and Deinonychosauria. The analysis further resulted in a sister group relationship between *Confuciusornis* and *Microraptor*, which is generally considered to be a basal dromaeosaur (*26*). Although this particular result may be due to the limited sampling of avian taxa, the presence of a deinonychosaurian key feature (a hyperextendible second toe) and the absence of two avian key features [a triradiate palatine (*3*) and a fully reversed first toe] in *Archaeopteryx* challenges the monophyly of Aves as currently recognized. There are no significant derived characters that are exclusively shared by *Archaeopteryx* and more typical avians such as *Confuciusornis* but are absent in basal deinonychosaurs such as *Microraptor*. The latter and *Confuciusornis* also share a number of derived features that are absent in *Archaeopteryx*, including ossified uncinate processes (optimized as a synapomorphy of the clade including *Confuciusornis* and deinonychosaurs in our analysis), an ulna that is much wider than the radius [not included in the matrix of (*26*)], and a plantarly situated first metatarsal (recovered as a synapomorphy of *Confuciusornis* + *Microraptor* in our analysis) (*26*). Thus Aves, if defined as the clade including *Archaeopteryx* and modern birds, may actually include taxa hitherto referred to as "deinonychosaurs" (*24*), some of which had fully developed avian-type wing feathers (*27*).

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Stem Cell Self-Renewal Controlled by Chromatin Remodeling Factors

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The self-renewing ability of a stem cell is controlled by its specialized microenvironment or niche, whereas epigenetic regulation of gene expression by chromatin remodeling factors underlies cell fate determination. Here we report that the adenosine triphosphate–dependent chromatin remodeling factors ISWI and DOM control germline stem cell and somatic stem cell self-renewal in the *Drosophila* ovary, respectively. The *iswi* mutant germline stem cells are lost rapidly because of defects in responding to bone morphogenetic protein niche signals and in repressing differentiation, whereas the *dom* mutant somatic stem cells are lost because of defective self-renewal. This work demonstrates that different stem cell types can use different chromatin remodeling factors to control cell self-renewal.

Extrinsic signals from niches are believed to control stem cell behavior, including self-renewal through interacting with intrinsic factors (*1*). However, it remains largely unclear how niche signals regulate their target gene expression in stem cells at the chromatin level. In the germarium at the tip of the *Drosophila* ovary, germline stem cells (GSCs) and somatic stem cells (SSCs) are attractive systems in which to study stem cells and their niches at the molecular and cellular level (*2*) (Fig. 1A). Terminal filament cells and cap cells at the tip of the germarium form a GSC niche, whereas posterior inner sheath cells in the middle of the germarium function as a SSC niche (*3*–*9*). GSCs can be reliably identified by their location (contact with cap cells), size, and spherical spectrosome (Fig. 1A). A GSC or SSC divides to generate two daughters: One daughter remains anchored to niche cells through Asp-Glu (DE)–cadherin–mediated cell adhesion and retains stem cell identity (*10*), whereas the other daughter moves away from niche cells and differentiates (Fig. 1A). Decapentaplegic (Dpp) and Hedgehog (Hh) are important for GSC self-renewal (*4*, *7*), whereas Hh and Wingless are essential for SSC self-renewal (*4*, *5*, *9*, *11*). In this study, we showed that adenosine triphosphate (ATP)–dependent chromatin remodeling factors control stem cell self-renewal by regulating responses to niche signals.

Chromatin remodeling factors are involved in maintaining chromatin structures and modulating gene expression in organisms ranging from yeast to humans (*12*). In *Drosophila*, five ATP-dependent remodeling factors, Brahma (BRM), Imitation SWI (ISWI), Domino (DOM), Kismet (KIS) and dMi-2, sharing a SNF2-related domain and a DEAD/DEAH-box helicase domain, appear in distinct protein complexes to control different gene activity in a variety of developmental processes (*13*–*17*). For example, ISWI plays a global role in chromatin compaction and transcriptional regulation (*17*–*19*); DOM, highly related to a chromatin remodeler SWR1 involved in exchanging histone variants, functions as a transcriptional repressor by interfering with the chromatin structure (*13*). *dom* and *iswi* are known to be required for normal oogenesis, but their roles in GSC and SSC regulation have not been determined (*13*, *19*). In this study, we focused on investigating the roles of *dom* and *iswi* in GSC and SSC regulation.

To analyze potential ISWI and DOM functions in ovarian stem cells, we characterized their expression in the germarium using available antibodies. The ISWI protein is present at high levels in the nuclei of all cell types in the germarium, including GSCs and SSCs (Fig. 1B). Although two DOM isoforms, DOM-A and DOM-B, are generally expressed, DOM-B is present in GSCs at higher levels than in other cells of the germarium (Fig. 1C). Because SSCs cannot be reliably identified by their location, morphology, or molecular markers, we used the MARCM (mosaic analysis with a repressible cell marker) system to generate green fluorescent protein (GFP)–marked SSCs for examining DOM-B expression (*20*). The positively marked SSCs can be readily identified on the basis of their ability to remain in the same position and continuously generate marked follicle cells (*6*, *9*, *21*). Indeed, DOM-B is present in the nuclei of SSCs at low levels (Fig. 1, C and D). These results suggest that DOM and ISWI could potentially have roles in GSC and SSC regulation.

To test whether *dom* and *iswi* are required for maintaining GSCs, we applied the Flipase (FLP)–mediated mitotic recombination technique to generate marked mutant GSCs and examined the loss rates of those marked mutant GSCs according to our published experimental procedures (*7*, *10*). The marked mutant GSCs were identified by their absence of LacZ staining and the presence of an anteriorly anchored spectrosome (Fig. 2A). dom^1, dom^3, $iswi^1$, and $iswi^2$, representing strong loss-of-function mutations, were used to generate marked mutant GSC clones (*13*, *19*). In contrast to the loss of 35% of the marked wild-type control GSC clones during the 2-week period, ranging from 3 days after clone induction (ACI) to 17 days ACI, about 99 and 96% of the marked mutant $iswi^1$ and $iswi^2$ GSC clones were lost during the same period, respectively, indicating that *iswi* is

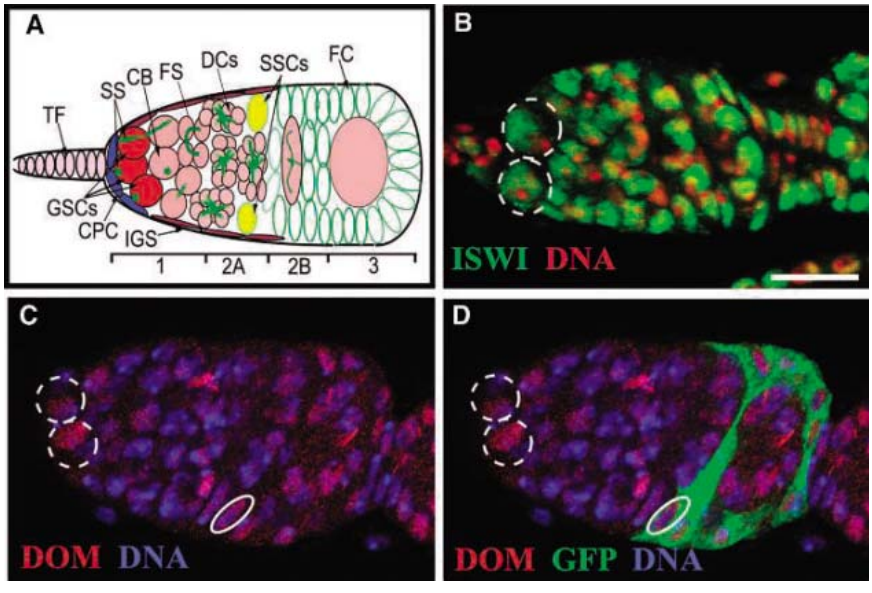

Fig. 1. ISWI and DOM are expressed in *Drosophila* ovarian GSCs and SSCs. (**A**) A schematic diagram showing a cross section of a *Drosophila* germarium. 1, 2A, 2B, and 3 indicate different regions of the germarium. All the images shown in all the figures represent one confocal section. (**B**) A germarium showing a uniform expression pattern throughout, including GSCs (dashed circles) and presumably SSCs. (**C** and **D**) A germarium showing that GSCs (dashed circles) express DOM at the highest level and that a GFP-marked SSC (solid circles) expresses DOM at a lower level. Abbreviations: TF, terminal filament; CPC, cap cells; IGS, inner sheath cells; SS, spectrosome; CB, cystoblast; FS, fusome; DCs, developing cysts; FC, follicle cells. Scale bar in (B), 10 μm.

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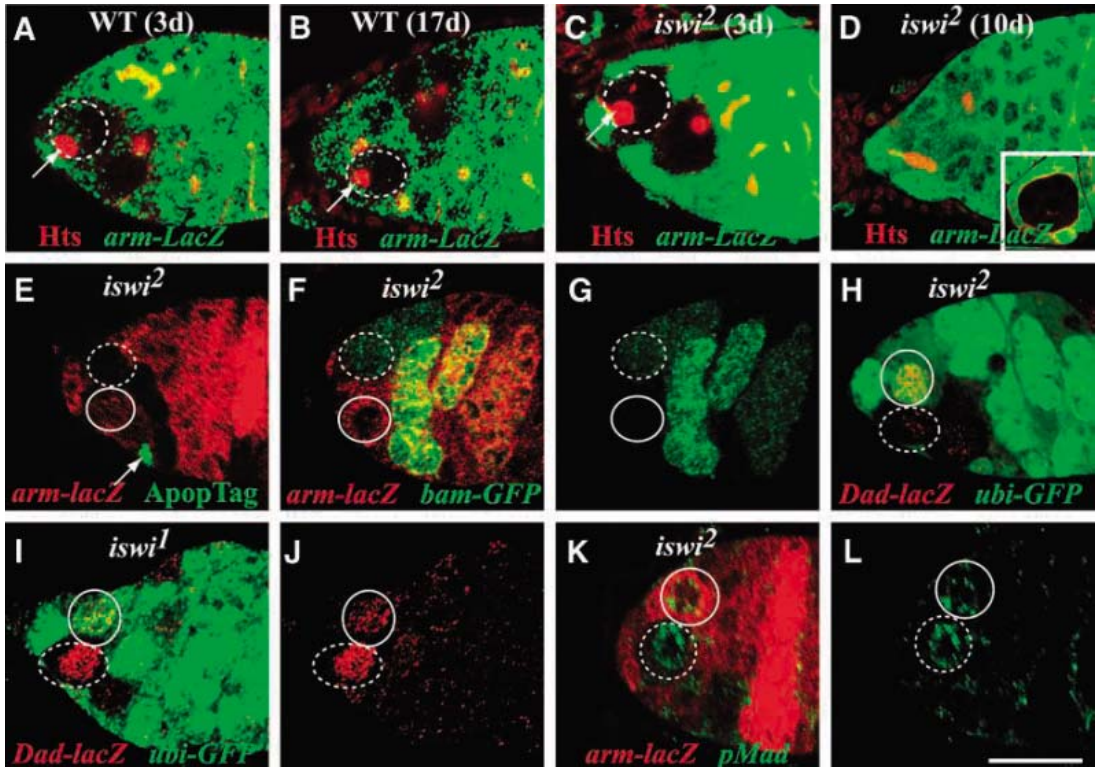

Fig. 2. ISWI is required for controlling GSC self-renewal by modulating responses to BMPs in the *Drosophila* ovary. In (A) to (F) and (K), marked GSCs are identified by the loss of *armadillo(arm)-lacZ* expression, whereas in (H) and (I), marked GSCs are identified by the loss of *ubiquitin (ubi)-GFP* expression. (**A** and **B**) Germarial tips carrying a 3-day-old (3d) (A) and a 17-day-old (B) marked wild-type GSC (dashed circles), in which the spectrosome is indicated by an arrow. (**C** and **D**) Germarial tips showing a 3-day-old *iswi*^{2} mutant clone [dashed circle in (C)] and a lost *iswi*^{2} mutant clone evidenced by the presence of a mutant egg chamber (inset) 10 days ACI. (**E**) A germarial tip showing an ApopTag-negative marked *iswi*^{2} mutant (dashed circle) and unmarked wild-type (solid circle) GSCs and a dying IGS cell (arrow). (**F** and **G**) A germarial tip showing that a marked *iswi*^{2} mutant GSC (dashed circles) but not an unmarked wild-type GSC (solid circles) up-regulates *bam-GFP* expression. (**H**) A germarial tip showing that a marked *iswi*^{2} mutant GSC (dashed circle) down-regulates *Dad-lacZ* expression in comparison with an unmarked wild-type GSC (solid circle). (**I** and **J**) A germarial tip showing that a marked *iswi*^{1} mutant GSC (dashed circles) up-regulates *Dad-lacZ* expression in comparison with an unmarked wild-type GSC (solid circles). (**K** and **L**) A germarial tip showing that both a marked *iswi*^{2} mutant GSC (dashed circles) and an unmarked wild-type GSC (solid circles) have comparable levels of nuclear pMAD. Scale bar in (L), 10 μm.

required for GSC maintenance (Table 1). Consequently, most of the marked control GSC clones that were detected 3 days ACI remained in their niches 17 days ACI (Fig. 2, A and B), whereas the majority of the marked mutant *iswi* GSC clones detected 3 days ACI were not present in their niches 17 days ACI (Fig. 2, C and D). Consistent with the idea that *iswi* mutations are responsible for the loss of the marked *iswi* mutant GSCs, a transgene *ISWI-HA*, which can fully rescue the lethality of both *iswi* mutants (*19*), also rescued the loss phenotype of those marked *iswi* mutant GSCs (Table 1). The marked mutant *dom*^{1} and *dom*^{3} GSC clones were lost similarly to the marked control clones, indicating that *dom* plays a minor, if any, role in GSC maintenance (Table 1). However, *dom* and *iswi* mutant oocytes formed normally but completely arrested at mid-oogenesis, which explains their requirements for normal oogenesis (*13*, *19*). These results demonstrate that *iswi* is required for maintaining GSCs.

To investigate whether *dom* and *iswi* are required for normal GSC division, we determined relative division rates for *dom* and *iswi* mutant GSCs by quantifying the average number of cysts produced by a marked mutant GSC, divided by the average number of cysts produced by a marked wild-type GSC (*10*). The marked *dom* mutant GSCs had a relative division rate close to 1.0, indicating that *dom* is not required for GSC division (Table 1). In contrast, the marked *iswi*^{1} and *iswi*^{2} mutant GSCs had much smaller relative division rates, 0.37 and 0.40, respectively. This result demonstrates that *iswi* is also required for stimulating GSC division.

Table 1. ISWI and DOM are required for maintaining GSCs and SSCs in the *Drosophila* ovary, respectively. nd, not determined.

Genotypes	Marked GSC clones				Marked SSC clones		
	3 days	10 days	17 days	Relative division rate	3 days	10 days	17 days
Wild type	33.6%* (244)	26.4% (182)	22.1% (164)	1.00† (37)	41.0% (244)	31.3% (182)	25.6% (164)
iswi^1	21.3% (338)	4.2% (471)	0.2% (470)	0.37 (13)	41.8 % (492)	20.8% (471)	11.7% (470)
iswi^1 and *ISWI-HA*‡	31.2% (247)	29.5% (285)	27.6% (217)	0.90 (15)	nd	nd	nd
iswi^2	34.3% (492)	11.0% (629)	1.4% (576)	0.40 (27)	32.5% (492)	30.7% (629)	18.4% (576)
iswi^2 and *ISWI-HA*	41.0% (210)	35.6% (225)	29.0% (176)	0.88 (21)	nd	nd	nd
dom^1	38.4% (594)	30.1% (721)	15.5% (689)	1.06 (50)	40.6% (594)	11.5% (721)	3.5% (689)
dom^3	31.6% (263)	27.0% (371)	26.6% (357)	0.97 (44)	35.4% (263)	12.7% (371)	2.0% (357)

*The percentage of germaria carrying marked GSCs for a given genotype is determined by the number of germaria carrying one or more marked GSCs divided by the number of total germaria shown in parentheses. †The relative division rate for a marked GSC is determined by the average number of marked cysts generated by a marked GSC divided by the average number of unmarked cysts generated by an unmarked GSC. The number of total marked GSCs examined is shown in parentheses. ‡*ISWI-HA* is a transgene.

iswi has been implicated in the regulation of cell survival in other developmental processes (*19*). Thus, we used the ApopTag cell death assay to determine whether marked *iswi* mutant GSCs undergo apoptosis. After 164 *iswi*^{2} mutant GSCs were examined, none of them were apoptotic, indicating that the loss of *iswi* mutant GSCs is likely not due to apoptosis but results from differentiation (Fig. 2E). These results suggest that ISWI regulates GSC self-renewal but not survival.

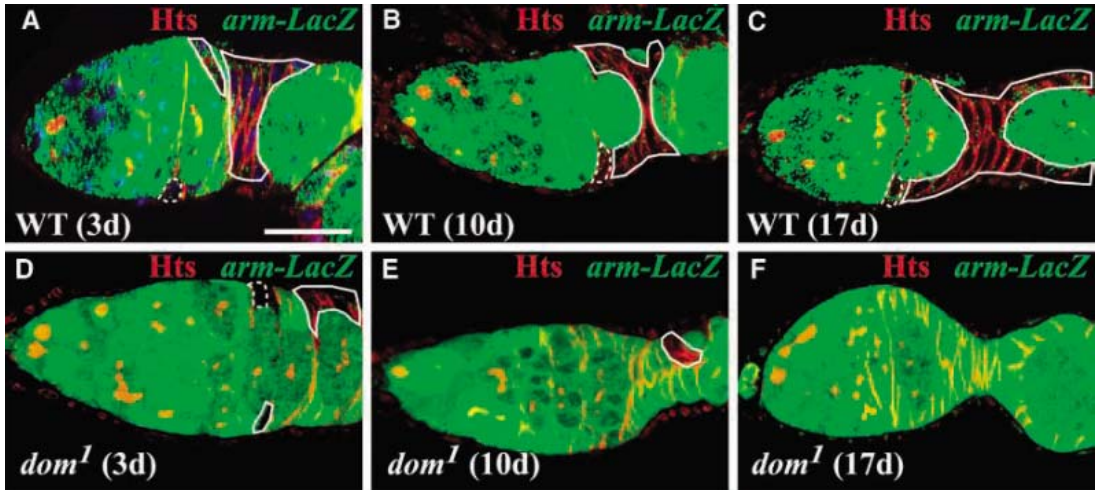

Fig. 3. DOM is required for SSC maintenance. Putative marked *lacZ*-negative SSCs are indicated by dashed lines; their marked progeny are indicated by solid lines. (**A** to **C**) Germaria carrying a 3-day-old (A), 10-day-old (B), and 17-day-old (C) marked wild-type SSC clone. (**D**) A germarium carrying a 3-day old marked dom^1 mutant SSC clone. (**E** and **F**) Germaria showing loss of marked mutant dom^1 SSC clones, evidenced by the presence of mutant follicle cells outside the germarium (E) or in a late egg chamber (F). Scale bar in (A), 10 μm.

Two bone morphogenetic protein (BMP) niche signals, Dpp and Gbb, control GSC self-renewal by producing phosphorylated MAD (pMAD), activating *Dad* transcription, and repressing the expression of the differentiation-promoting *bag of marbles* (*bam*) gene in GSCs (*22*–*24*). To test the possibility that *iswi* is required in GSCs for properly responding to BMP signals, we determined pMAD expression, *Dad* transcription, and *bam* repression in marked *iswi* mutant and unmarked wild-type GSCs of the same germaria. The *Dad-lacZ* line and the *bam-GFP* line were used to recapitulate *Dad* and *bam* gene expression (*22*–*24*). We found that 35 and 39% of the 1-week-old marked $iswi^1$ (63 marked GSCs examined) and $iswi^2$ (81 marked GSCs examined) mutant GSCs up-regulated *bam-GFP* expression in comparison with their neighboring unmarked wild-type GSCs (144 GSCs examined), which showed no *bam-GFP* expression, indicating that *iswi* is required for BMP signaling–mediated *bam* transcriptional repression in GSCs (Fig. 2, F and G). In addition, 41.0 and 44.6% of the 1-week-old marked $iswi^1$ (139 marked GSCs examined) and $iswi^2$ (130 marked GSCs examined) mutant GSCs showed misregulated *Dad-lacZ* expression (down-regulation and up-regulation) in comparison with their neighboring wild-type GSCs of the same germaria (Fig. 2, H to J), suggesting that *iswi* is required for proper BMP signaling–mediated transcriptional activation in GSCs. However, the pMAD levels in all the mutant *iswi* GSCs examined (41 $iswi^2$ mutant GSCs) remained similar to those in the wild-type GSCs in the same germaria, suggesting that *iswi* is dispensable for BMP signal transduction in GSCs (Fig. 2, K and L). These results show that ISWI facilitates the proper interpretation of BMP signaling in GSCs. Furthermore, we showed that ISWI protein expression levels in GSCs are not regulated by BMP signaling (*25*) (fig. S1). Together, these results demonstrate that ISWI is involved in BMP signaling–mediated gene repression and activation in GSCs.

We then generated marked *dom* and *iswi* mutant SSC clones to investigate their role in SSC regulation in the same way as we examined the marked GSCs. A SSC marked by loss of *arm-lacZ* expression was identified by its location (in the middle of the germarium) and its ability to generate marked follicle cells in and outside of the germarium. As a control, 37.5% of the marked wild-type SSC clones detected 3 days ACI were lost 17 days ACI, whereas most of them remained (Fig. 3, A to C, and Table 1). During the same period, 43.3 and 72.0% of the marked $iswi^1$ and $iswi^2$ mutant SSC clones were lost, respectively, indicating that *iswi* plays a minor role in maintaining SSCs (Table 1). In contrast, 91.4 and 94.4% of the marked dom^1 and dom^3 mutant SSC clones detected 3 days ACI were lost 17 days ACI, indicating that *dom* is essential for maintaining SSCs (Fig. 3, D to F, and Table 1). Furthermore, we used the ApopTag cell death assay and overexpression of a cell death inhibitor, *p35*, to show that mutations in *dom* did not increase apoptosis in the marked *dom* mutant SSCs (a total of 66 mutant dom^1 and dom^3 SSCs examined) and that p35 overexpression in the marked *dom* mutant SSC clones failed to prevent their loss (*25*) (table S1 and fig. S2). Our results demonstrate that *dom* specifically controls SSC self-renewal but not survival.

This study reveals that ATP-dependent chromatin remodeling factors are required to control stem cell self-renewal. ISWI controls GSC self-renewal at least in part through regulating responses of stem cells to BMP niche signals. Furthermore, we also show that DOM is essential only for SSC self-renewal, whereas ISWI is essential only for GSC self-renewal. This suggests that different stem cell types depend on different chromatin remodeling factors to control their self-renewal. Recent studies show that Polycomb-like *bmi-1* is required for maintaining the self-renewal of blood stem cells and neural stem cells (*26*, *27*). Because extensive knowledge about Polycomb and other chromatin factors already exists regarding *Drosophila*, further studies on the roles of ISWI and DOM in the regulation of *Drosophila* ovarian stem cells would provide insight into how chromatin remodeling factors control stem cell self-renewal. Given an evolutionarily conserved function of SWI/SNF chromatin remodeling complexes, their function in stem cell regulation is likely to be conserved evolutionarily in mammals, including humans.

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Supporting Online Material

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Materials and Methods
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Restoration of Auditory Nerve Synapses in Cats by Cochlear Implants

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Congenital deafness results in abnormal synaptic structure in endings of the auditory nerve. If these abnormalities persist after restoration of auditory nerve activity by a cochlear implant, the processing of time-varying signals such as speech would likely be impaired. We stimulated congenitally deaf cats for 3 months with a six-channel cochlear implant. The device used human speech-processing programs, and cats responded to environmental sounds. Auditory nerve fibers exhibited a recovery of normal synaptic structure in these cats. This rescue of synapses is attributed to a return of spike activity in the auditory nerve and may help explain cochlear implant benefits in childhood deafness.

In deaf humans, cochlear implants have restored hearing for many but not all recipients. The clinical consensus is that oral language development before deafness leads to the best outcomes. Among congenitally deaf children, the younger the age of implant activation, the better the aural language results (*1*). These clinical experiences imply that uncorrected congenital deafness introduces irreversible abnormalities in the developing central nervous system. In mammalian models of congenital deafness, the synaptic structure of auditory nerve endings is abnormal (*2–4*). Could the status of auditory nerve synapses represent an important link to the success or failure of cochlear implants?

One defect in the auditory nerve of congenitally deaf animals occurs at a large axosomatic ending of myelinated auditory nerve fibers called the endbulb of Held (*2–4*). Endbulbs have a calyxlike appearance that is formed from the main axon as several gnarled branches that arborize repeatedly to enclose the postsynaptic cell in a nest of en passant swellings and terminal boutons (*5*). They transmit signals from the auditory nerve fiber to the postsynaptic cell with a high degree of fidelity (*6*, *7*) and are implicated in the pathways that process the temporal features of sound (*8*, *9*). Congenitally deaf animals exhibit endbulbs with marked reduction in their branching (*3*, *10*). Moreover, they contain fewer synaptic vesicles, and postsynaptic densities are longer and straighter (*2*, *3*). These structural abnormalities have been associated with transmission irregularities at the synapse of endbulbs (*11*, *12*) that may underlie loss of temporal resolution of midbrain (*13*) and cortical (*14*) neurons in neonatally deafened cats.

Electrical stimulation in deaf cats has resulted in improvements in temporal processing at the level of the auditory cortex (*15*) and inferior colliculus (*16*). Our goal was to test whether such benefits might be mediated through "rescue" of endbulb synapses. We sought to determine what happened to these synapses when spike activity was reintroduced to the auditory nerve. The synaptic status in implanted deaf animals may yield insights into the beneficial intervention effects reported for children who undergo implantation at a young age (*17–19*).

We compared auditory nerve endings in the cochlear nucleus of congenitally deaf cats fitted with a cochlear implant (n = 3) with those of normal-hearing cats (n = 3) and congenitally deaf cats (n = 4). Complete deafness of the auditory nerve for the congenitally deaf cats was verified by the absence of acoustically evoked brainstem responses. Congenitally deaf cats were implanted at 2.5, 3, and 5 months of age. Following a 2- to 3-week recovery, implant hardware was programmed and the cats received stimulation for nearly 8 hours per day, 5 days per week. Electrically evoked compound action potentials (ECAPs) (Fig. 1) and behavioral responses were monitored monthly to verify continued function of each electrode and to ensure that signals were being delivered to the nerve (*20*). We were confident that environmental sounds had biological importance to the animals, because we could routinely "call" implanted cats for a food reward. After 3 months of stimulation, cats were euthanized by overdose of sodium pentobarbital and transcardially perfused with buffered fixative, and brain tissue was harvested for light and electron microscopic analysis. Tissue sections of the cochlear nucleus were histologically prepared using standard procedures for examination with an electron microscope (*2*).

In the anteroventral cochlear nucleus, auditory nerve synapses have a readily identifiable ultrastructural appearance (*21*, *22*). Endbulbs contain clear, round synaptic vesicles (50 nm in diameter) and exhibit multiple asymmetric membrane thickenings with the somata of spherical bushy cells in the cochlear nucleus (Fig. 2). These membrane thickenings are called postsynaptic densities, contain transmitter receptors, and are assumed to represent the synaptic release sites. They are punctate, marked by dense material in the cytoplasm, and arched outward into the presynaptic endbulb. When individual synapses were followed through serial sections and reconstructed using computer software, their discrete nature was revealed by observing the surface of the postsynaptic

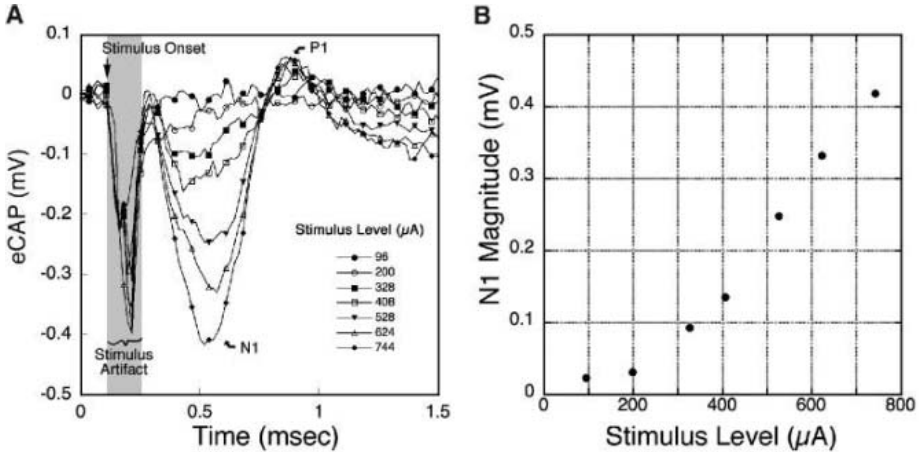

Fig. 1. (**A**) Recording of ECAP where stimulation was delivered by electrode 1 and recordings were collected from electrode 3. Electrode 1 is the most apical electrode. Stimulus onset and stimulus artifact are indicated, as are N1 and P1 potentials. This ECAP was representative of our cats and exhibited waveforms and peak latencies similar to what has been shown for humans (*20*). (**B**) N1-P1 amplitude as a function of stimulus level. Note the growth in response with increasing stimulus levels. These data suggest that electrical signals from the cochlear implant are entering the brain via the auditory nerve.

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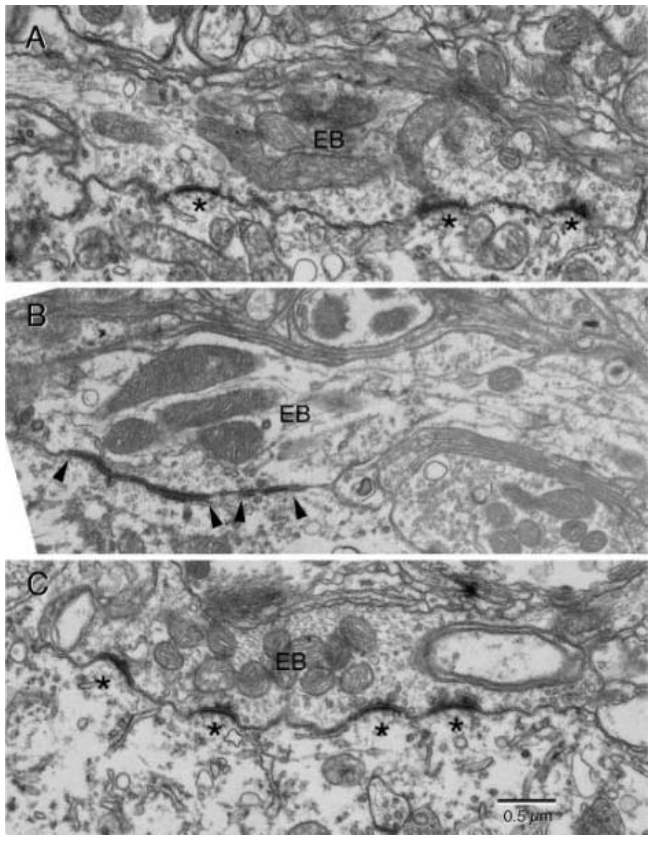

Fig. 2. Electron micrographs of endbulb (EB) synapses from (**A**) a normal-hearing cat, (**B**) a congenitally deaf cat that was untreated, and (**C**) a congenitally deaf cat that received 3 months of electrical stimulation from a cochlear implant. All micrographs were collected from cats that were 6 months of age. The hearing and treated cats exhibit synapses that are punctate, curved, and accompanied by nearby synaptic vesicles (asterisks). In contrast, the synapses from untreated deaf cats are large, flattened, and mostly void of synaptic vesicles (arrowheads). Scale bar, 0.5 μm.

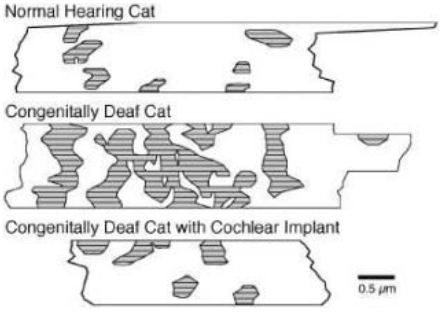

Fig. 3. Postsynaptic densities from endbulbs of Held, indicative of release sites, were reconstructed through serial sections using three-dimensional software and then rotated. These views present the surface of the bushy cell membrane that lies under the auditory nerve ending. The shaded and lined regions represent the reconstructed synapses. Each horizontal line indicates a single ultrathin section. The congenitally deaf cats exhibited hypertrophied synapses, whereas the stimulated deaf cats exhibited synapses having normal shapes and distributions.

membrane (Fig. 3). The reconstructed area of individual postsynaptic densities from normal-hearing cats averaged 0.127 ± 0.071 μm^2 (n = 129). In contrast, the endbulb synapses of congenitally deaf cats exhibited distinct differences. There was a reduction in the number of synaptic vesicles, and the postsynaptic densities appeared flattened, thicker, and longer (Fig. 2). When individual postsynaptic densities were reconstructed through serial sections, they were hypertrophied (Fig. 3), averaging 0.301 ± 0.398 μm^2 (n = 152).

In stimulated deaf cats, endbulb synapses resembled those from normal-hearing cats (Fig. 2). The full complement of synaptic vesicles was present, and the curvature of the typical normal postsynaptic density had returned. When the postsynaptic densities from the implanted cats were reconstructed through serial sections, they were normal in size (0.093 ± 0.078 μm^2, n = 58) and spatial distribution (Fig. 3). Analysis of variance revealed that there was no significant difference between the size of postsynaptic densities from the auditory nerve fibers of normal-hearing and implanted cats, whereas those from congenitally deaf cats were significantly larger ($P < 0.001$). If these cats had not received such treatment, their synapses would have remained pathologic (*2*).

The importance of neural activity for normal development and function of sensory systems is well documented (*23*–*27*). There is growing evidence to suggest that prosthetic stimulation serves as an adequate substitution. Induced neural activity by electrical stimulation in deaf cats has been reported to restore cell size in the region of the cochlear nucleus innervated by endbulbs (*28*–*30*), an effect presumably mediated by the restored synapses. Without stimulation, the cells were shrunken (*31*, *32*). These data establish that the morphology of central auditory synapses is malleable not only to deprivation but also to stimulation.

The functional importance of synaptic recovery in auditory nerve fibers is that temporal processing is improved in the chronically stimulated cats. Preservation of the "timing pathway" through the endbulb–bushy cell circuit would support the precise transmission of temporal cues within the auditory signal. Because the cochlear nucleus gives rise to all ascending auditory pathways, the normalization of synapses is hypothesized to enable the faithful transmission of auditory signals throughout the system. Temporal resolution of neurons in the inferior colliculus demonstrated impaired frequency following of neurons in neonatally deafened cats. This impaired frequency following improved after electrical stimulation of the cochlea (*16*). The critical nature of temporal resolution in facilitating speech recognition is underscored by studies that show speech recognition based on temporal cues while spectral content is systematically degraded (*33*). From the cochlear nucleus, high-fidelity temporal features of sound can be used to mediate more complex functions occurring in auditory cortex (*15*, *34*, *35*). It is logical to infer that deafness results in synaptic abnormalities in auditory nerve fibers of congenitally deaf humans, similar to those found in other mammals. We hypothesize that the changes observed after cochlear implantation at this crucial synapse enable the development of integrative and cognitive brain functions reflected in aural and oral communication in deaf children (*1*, *17*–*19*, *36*, *37*).

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A Role for the Phagosome in Cytokine Secretion

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Membrane traffic in activated macrophages is required for two critical events in innate immunity: proinflammatory cytokine secretion and phagocytosis of pathogens. We found a joint trafficking pathway linking both actions, which may economize membrane transport and augment the immune response. Tumor necrosis factor α (TNFα) is trafficked from the Golgi to the recycling endosome (RE), where vesicle-associated membrane protein 3 mediates its delivery to the cell surface at the site of phagocytic cup formation. Fusion of the RE at the cup simultaneously allows rapid release of TNFα and expands the membrane for phagocytosis.

In response to a microbial challenge, activated macrophages initiate multiple actions, including the secretion of proinflammatory cytokines and the phagocytosis of microorganisms (*1*). Both of these actions require substantial up-regulation of protein trafficking and deployment of membrane to the cell surface. TNFα, the earliest and most potent proinflammatory cytokine released, has an essential role in immunity but also plays a causative role in inflammatory disease (*2*). The secretory pathway for the trafficking of the newly synthesized transmembrane form of TNFα to the cell surface is unknown. Our approach has been to identify specific vesicular machinery that functions in this pathway (*3*, *4*). Soluble *N*-ethylmaleimide–sensitive factor (NSF) attachment receptor (SNARE)–mediated fusion of vesicular carriers is a requirement of this and other trafficking pathways (*5*). Q-SNARE complexes on the Golgi (syntaxin 6–syntaxin 7–Vti1b) and at the cell surface [syntaxin 4–SNAP23 (synaptosomal-associated protein of 23 kD)] function in and are rate-limiting for TNFα trafficking and secretion (*3*, *4*). A microarray

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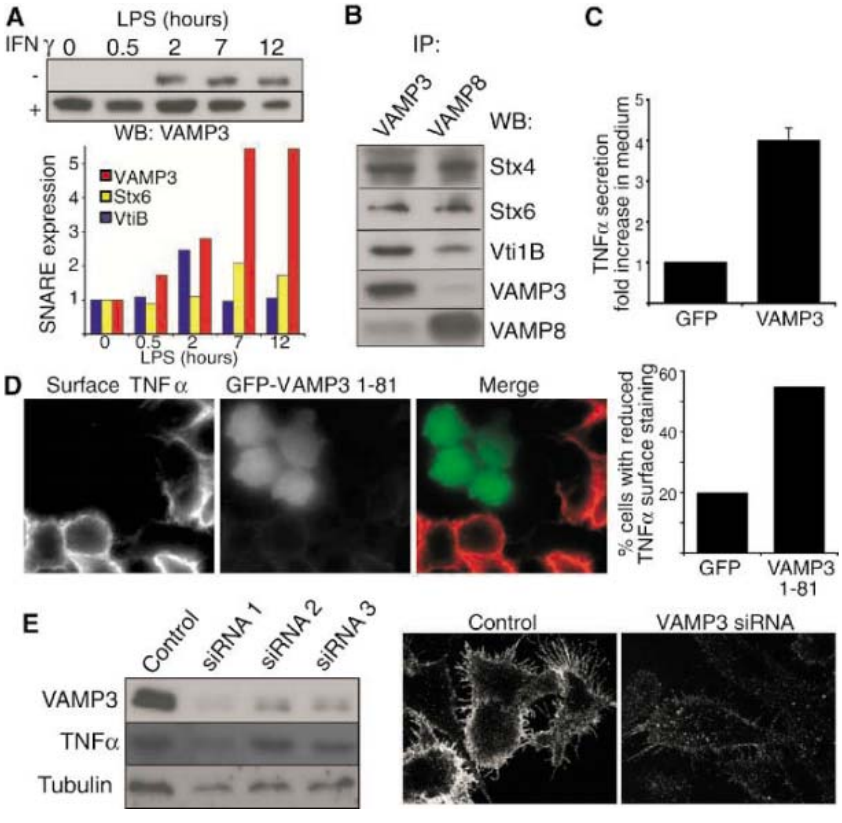

Fig. 1. VAMP3 up-regulation and function as the R-SNARE for TNFα secretion. (**A**) VAMP3 protein expression: up-regulation by LPS compared with other relevant SNAREs and in IFN-primed cells. WB, Western blotting. (**B**) SNARE partners coimmunoprecipitated (IP) with VAMP3 or VAMP8 are detected by immunoblotting. (**C**) TNFα secreted into media of transfected cells (GFP alone or GFP-VAMP3) measured by enzyme-linked immunosorbent assay (ELISA). Error bar indicates SEM. (**D**) Surface TNFα stained on unpermeabilized, TACE inhibitor–treated cells. Bar graph shows proportion of cells expressing GFP alone or GFP-VAMP3(1-81) mutant with >2-fold reduction in surface TNFα-staining expression. (**E**) Targeted knockdown of VAMP3 protein using three different specific siRNAs (left), and surface staining of TNFα in control (no siRNA) or siRNA-transfected cells (right) (*23*).

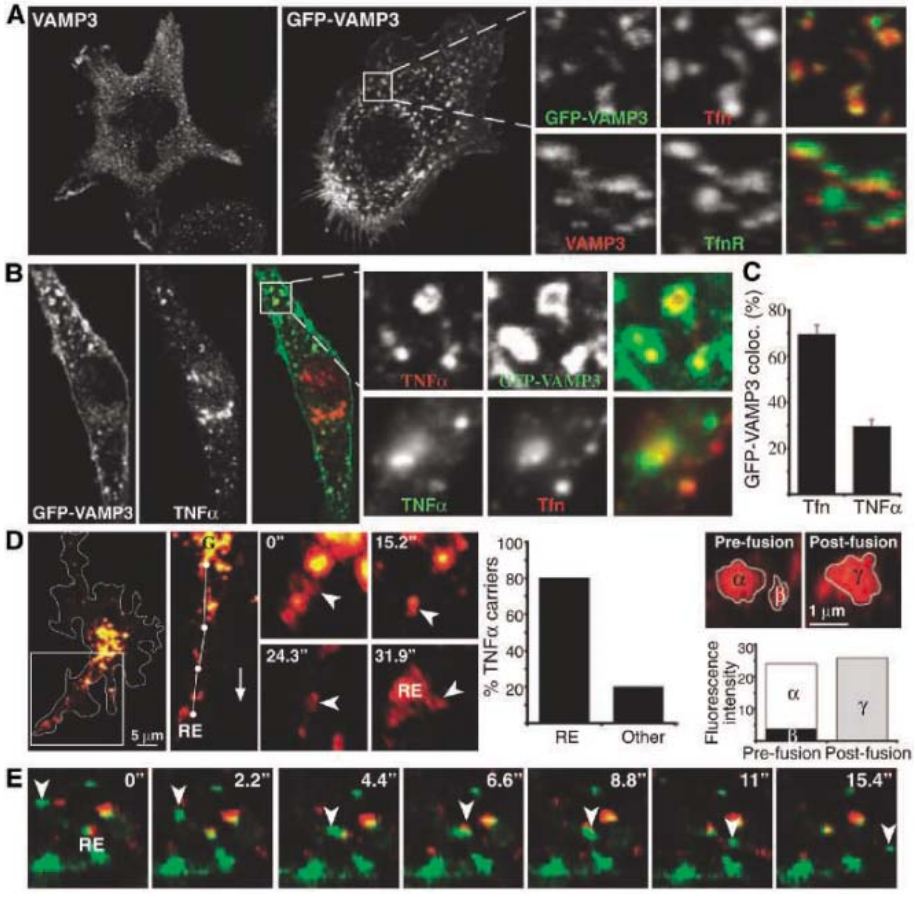

Fig. 2. TNFα trafficking to the cell surface via the RE. (**A**) In fixed cells, immunostaining of VAMP3 and GFP-VAMP3 colocalized with staining of Tfn (enlarged from box) and with Tfn receptor (TfnR). (**B**) Immunostaining and colocalization of TNFα with GFP-VAMP3 in REs (enlarged from box) and with Tfn. (**C**) Proportion of Tfn or TNFα immunostaining colocalized with GFP-VAMP3. Error bars indicate SEM. (**D**) GFP-TNFα imaged in live cells is concentrated in the Golgi (G) region and in carriers (~250-nm diameter; arrow) moving along a path (sequential fixed points enlarged; arrowheads) toward a RE (1.5-μm diameter) in the cell periphery. (Right) Resulting fusion (γ) of a GFP-TNFα carrier (β) with the RE (α) is shown and quantified by fluorescence intensity of each compartment. Middle bar graph shows GFP-TNFα carriers (%) imaged leaving the Golgi region in live cells delivered to a RE compared with other destinations. (**E**) Live imaging of a cell expressing GFP-TNFα (green) after uptake of labeleled Tfn (red). Sequence of images showing movement of a Golgi-derived GFP-TNFα carrier (arrowheads) moving to and fusing with a RE (at 4.4 min) followed by exit of another carrier (at 11 min) from this RE.

screen identified vesicle-associated membrane protein 3 (VAMP3) as a lipopolysaccharide (LPS)-regulated SNARE protein (table S1). Interferon-γ (IFN-γ)-primed, LPS-stimulated macrophages synthesize more TNFα and secrete it faster than cells activated with LPS alone (*6*). VAMP3 is also up-regulated by IFN-γ priming, and its expression is modulated in temporal and quantitative fashions to match relevant Q-SNARE expression and TNFα trafficking (Fig. 1A).

By coimmunoprecipitation, VAMP3 interacted with syntaxin 4, syntaxin 6, and Vti1b (Fig. 1B), indicating its potential as the cognate R-SNARE for the Golgi-associated and cell-surface Q-SNARE complexes (*3*). Trafficking in a variety of cells, including macrophages, can be enhanced by overexpression of full-length SNAREs, whereas overexpression of the SNARE cytoplasmic domains competitively inhibits and blocks vesicle docking and fusion (*3*, *4*, *7*). Overexpression of green fluorescent protein (GFP)–tagged VAMP3 in macrophages resulted in a fourfold increase in TNFα secretion (Fig. 1C). In contrast, expression of the inhibitory cytoplasmic domain of VAMP3 (GFP-VAMP3 amino acids 1 to 81) [but not of syntaxin 2, an irrelevant SNARE (*3*, *4*)] blocked the delivery of TNFα to the cell surface (Fig.

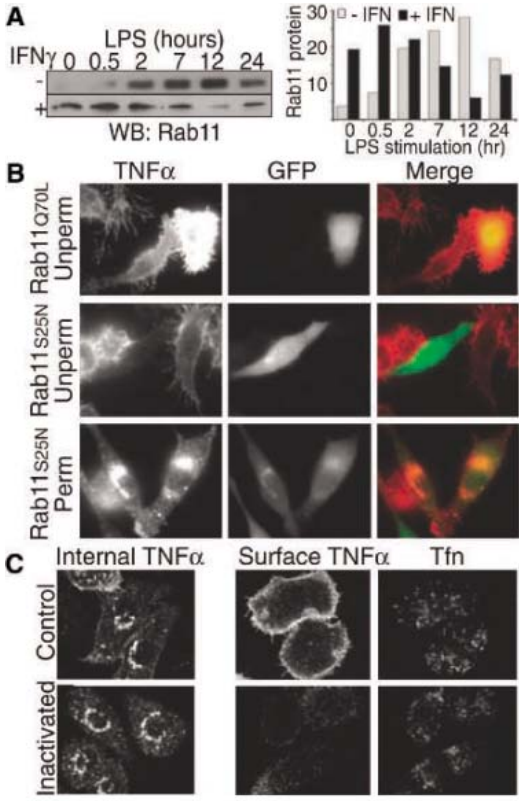

Fig. 3. Recycling endosomes are required for TNFα trafficking. (**A**) LPS- and IFN-regulated expression of the RE protein Rab11. (**B**) Surface delivery and staining of TNFα in cells treated with LPS and TACE inhibitor and expressing Rab11 mutants. TNFα staining in the Golgi after cell permeabilization. (**C**) Surface delivery and staining of TNFα in cells after uptake of HRP-Tfn for enzymatic inactivation of the RE (*13*) and treatment with LPS and TACE inhibitor.

1D) without compromising newly synthesized TNFα at the level of the Golgi (*8*). Short interfering RNA (siRNA) knockdown of VAMP3 expression (by >80%) also inhibited delivery and reduced surface staining of TNFα without affecting its synthesis (Fig. 1E). Thus, VAMP3 acts as the functional and rate-limiting R-SNARE for the known Golgi and cell surface Q-SNAREs required for post-Golgi transport of TNFα.

In macrophages, endogenous VAMP3 is located on vesicular membranes and plasma membrane ruffles (Fig. 2A). GFP-VAMP3 is similarly distributed, and at higher expression amounts it is also on enlarged cytoplasmic organelles and is more widespread on the cell surface (Fig. 2A). The majority of vesicular VAMP3 or GFP-VAMP3 colocalized with internalized transferrin (Tfn), Tfn receptor (TfnR), or Rab11 as markers of the recycling endosome (RE) (*9*, *10*) (Fig. 2A and fig. S1). Notably, a proportion of TNFα (30%) in peripheral structures colocalized with GFP-VAMP3 (Fig. 2B) and with Tfn on the RE, suggesting an unexpected route for TNFα exocytosis. In live macrophages, GFP-TNFα was transported from the Golgi to peripheral structures identified as Tfn-positive REs en route to the cell surface (Fig. 2, D and E, and movies S1 to S3). This transport occurred in pleiomorphic carriers whose trajectories and behavior are consistent with post-Golgi carriers (*11*, *12*) (Fig. 2, D and E, and movies S1 to S3). Close analysis confirmed that carriers and the RE fuse (Fig. 2D), and that at least 80% of the TNFα carriers exiting the Golgi fused with REs, verifying the requirement for two SNARE-mediated fusion events in the post-Golgi trafficking of TNFα. Golgi-to-RE transport involves pairing of the syntaxin 6–syntaxin 7–Vti1b–Q-SNARE with the R-SNARE–VAMP3 on the RE, then VAMP3 pairs with the Q-SNARE (syntaxin 4–SNAP23) on the plasma membrane.

The LPS- and IFN-γ-induced up-regulation of another RE protein, Rab11 (Fig. 3A), mirrored the expression of other machinery required for TNFα trafficking (*3*). A constitutively active mutant of Rab11 expressed in macrophages increased cell surface delivery of TNFα, whereas dominant-negative Rab11 blocked TNFα cell surface delivery without affecting newly synthesized TNFα at the Golgi complex (Fig. 3B). The functional requirement for the RE as a transit station in TNFα exocytosis was further demonstrated by the use of a horseradish peroxidase (HRP) inactivation assay (*13*). Cell surface delivery of TNFα was effectively blocked by inactivation of REs, accumulating TNFα at the Golgi and en route (Fig. 3C). The RE in activated macrophages thus assumes a pivotal role as a hub for exocytic trafficking. Correspondingly, the RE is integral to polarized exocytosis in epithelial cells (*11*, *13*). How does this route serve to expedite the release of TNFα as an early response proinflammatory cytokine?

Activated macrophages increase their cell surface area for phagocytosis (*14*) by using extra membrane from endoplasmic reticulum (ER), lysosomes, and endosomes in SNARE-mediated fusion events (*10*, *15*–*18*), although the contribution from the ER is now controversial (*19*). We confirmed a role for VAMP3 in surface membrane expansion by fluorescence-activated cell sorter (FACS) analysis where overexpression of GFP-VAMP3 maximally increased the surface area of fluorescently labeled macrophages (fig. S2). Because VAMP3 function is also required for fully efficient phagocytosis of yeast (*16*, *20*), we next investigated the possible convergence of VAMP3 roles in phagocytosis and surface delivery of TNFα. Primed macrophages incubated with the yeast *Candida albicans* for 20 to 40 min were imaged live or after fixation. In the initial stages of phagocytosis (before closure), actin-rich phagocytic cups (*21*) were labeled for VAMP3 (*10*) and for TNFα (Fig. 4A). TNFα staining was not seen after internalization or in more mature phagosomes labeled with LAMP1 (*21*) (Fig. 4, A and B). Addition of a TNFα converting enzyme (TACE) inhibitor to block proteolytic release of surface TNFα revealed that newly delivered surface TNFα was highly concentrated in the phagocytic cups compared with the surrounding plasma membrane (Fig. 4C). This was not the case for an unrelated and evenly distributed plasma membrane marker, GFP-tagged K-ras C-terminal targeting motif (GFP-tK) (Fig. 4C), or a secreted protein apolipoprotein E (movie S6). TNFα was not delivered randomly to the cell surface but was specifically delivered to sites of phagocytic cup formation. The absence of surface and soluble forms of TNFα from mature phagolysosomes suggests that soluble TNFα is cleaved and released rapidly, before closure of the cup. VAMP3 and other relevant machinery were clustered at the delivery site, including syntaxin 4, part of the cell surface Q-SNARE complex required for

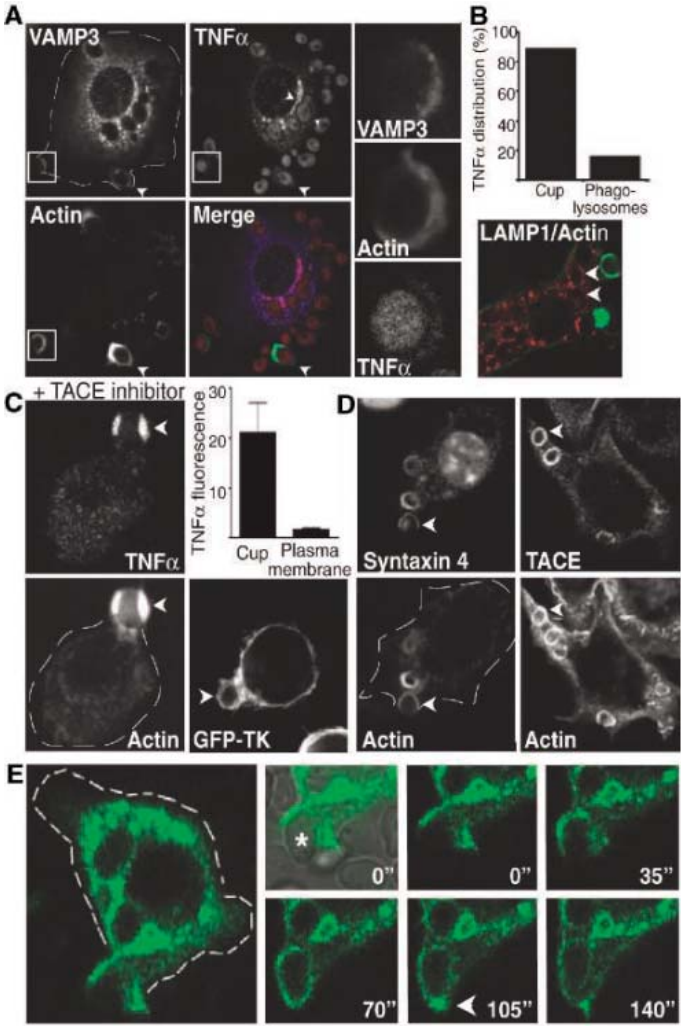

Fig. 4. RE delivers TNFα to newly forming phagocytic cups. (**A**) Primed macrophages were incubated with *C. albicans* (autofluorescing) and then fixed, permeabilized, and stained for VAMP3, TNFα, and F-actin. Boxed area enlarged to show staining at an actin-rich phagocytic cup. (**B**) Proportion of TNFα staining on phagocytic cups (actin-rich) compared with internalized phagosomes. Internalized mature phagosomes stain for LAMP1 but not F-actin. (**C**) Staining of TNFα at the actin-rich phagocytic cup enhanced in the presence of TACE inhibitor. Bar graph shows TNFα immunofluorescence in cups compared with rest of the plasma membrane. There is even distribution of another marker, GFP-tK, in transfected cells across all of the plasma membrane. Error bars indicate SEM. (**D**) Immunostaining for syntaxin 4 and TACE in actin-rich phagoctyic cups and phagososmes. (**E**) Live imaging of a primed macrophage expressing GFP-TNFα during phagocytosis (movie S4). Enlarged images show GFP-TNFα as it moves around the yeast (asterisk indicates phase contrast at 0 min) and culminates at the outermost point (arrowheads) after engulfment.

TNFα delivery (Figs. 4D and 1B), and TACE, the enzyme responsible for cleavage and release of soluble TNFα (*22*) (Fig. 4D). Lastly, we observed the movement of GFP-TNFα to the phagocytic cup during the internalization of *C. albicans* in live cells (Fig. 4E and movies S4 and S5). Membranes containing GFP-TNFα or GFP-VAMP3 were seen forming the initial phagocytic cup. As membrane moved around the yeast, engulfing and internalizing it, TNFα was concentrated in a patch on the outermost point of the cell surface from where it was cleaved and released (Fig. 4E, fig. S3, and movies S4 and S5). Thus, TNFα- and VAMP3-containing RE membranes translocate to the nascent phagocytic cup for SNARE-mediated fusion during the initial stages of phagocytosis. This presents both an unexpected site for cytokine secretion and a rapid and efficient mechanism for release of an early response inflammatory mediator. Having a single route for membrane flow to the cell surface via the RE that both delivers TNFα to the plasma membrane and expands the plasma membrane for phagocytic cup formation neatly combines two early actions of activated macrophages mounting an innate immune response.

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23. Materials and methods and supporting data are available on *Science* Online.

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ATP Signaling Is Crucial for Communication from Taste Buds to Gustatory Nerves

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Taste receptor cells detect chemicals in the oral cavity and transmit this information to taste nerves, but the neurotransmitter(s) have not been identified. We report that adenosine 5′-triphosphate (ATP) is the key neurotransmitter in this system. Genetic elimination of ionotropic purinergic receptors ($P2X_2$ and $P2X_3$) eliminates taste responses in the taste nerves, although the nerves remain responsive to touch, temperature, and menthol. Similarly, P2X-knockout mice show greatly reduced behavioral responses to sweeteners, glutamate, and bitter substances. Finally, stimulation of taste buds in vitro evokes release of ATP. Thus, ATP fulfils the criteria for a neurotransmitter linking taste buds to the nervous system.

Taste buds transduce chemical signals in the mouth into neural messages transmitted to gustatory nerve fibers of the facial and glossopharyngeal nerves. Despite recent progress in delineating the molecular mechanisms for taste transduction, the identity of the neurotransmitter from taste buds to the nerve fibers is unknown. Elucidation of the neurotransmitter between taste cells and nerve fibers is complicated by several factors. Each taste bud contains a variety of cell types, likely communicating with each other as well as with the gustatory nerve fibers. Hence, the mere presence of a potential transmitter substance in the taste bud does not imply that a particular substance is used to activate the gustatory nerve fibers.

Recent studies have suggested that serotonin [5-hydroxytryptamine (5-HT)] is released by taste buds (*1*) and acts on $5\text{-}HT_3$ receptors on the gustatory nerve fibers to convey the taste message (*2*). To test this hypothesis, we examined the taste behavior of $5\text{-}HT_{3A}$ knockout (KO) mice. The $5\text{-}HT_{3A}$ subunit is crucial for the function of both homomeric and heteromeric $5\text{-}HT_3$ receptors (*3*). These KO mice have depressed behavioral thresholds to pain, in which serotonin acts as both a peripheral activator and a central modulator (*4*). In contrast, the taste behavior of the $5\text{-}HT_{3A}$ KO mice is identical to that of wild-type controls for each taste quality tested (fig. S1). Thus, serotonin does not act on neural $5\text{-}HT_3$ receptors to transmit taste information from taste buds to the gustatory nerve.

Taste nerves express two ionotropic P2X receptor subunits ($P2X_2$ and $P2X_3$) (*5*), which suggests that adenosine triphosphate (ATP) may serve as a neurotransmitter in this system. $P2X_2$ and $P2X_3$ receptor subunits can form homomeric $P2X_2$, homomeric $P2X_3$, and heteromeric $P2X_{2/3}$ receptors (*6*). ATP acting on $P2X_2$ receptors (likely homomeric $P2X_2$) appears crucial to transmission in the carotid body, which has structural similarities to taste buds (*7*). In order to test whether the P2X receptors are required for transmission of taste information, we recorded neural responses to chemical stimulation of the oral cavity in $P2X_2/P2X_3$ double-knockout [$P2X_2/P2X_3^{Dbl-/-}$ (KO)] mice (*8*), C57BL/6J mice, and $P2X_2/P2X_3$ double wild-type [$P2X_2/P2X_3^{Dbl+/+}$ (WT)] mice. No taste-evoked activity was seen in either the chorda tympani or glossopharyngeal nerves of $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice. In contrast, stimulation by touch, temperature, and menthol solutions elicited robust neural responses (Fig. 1). Menthol activates fine-caliber somatosensory nerve fibers via direct action on neural transient receptor potential (TRP) channel receptors and does not involve taste buds (*9*). Thus, although the gustatory nerves of $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice are highly responsive to somatosensory stimuli, they are

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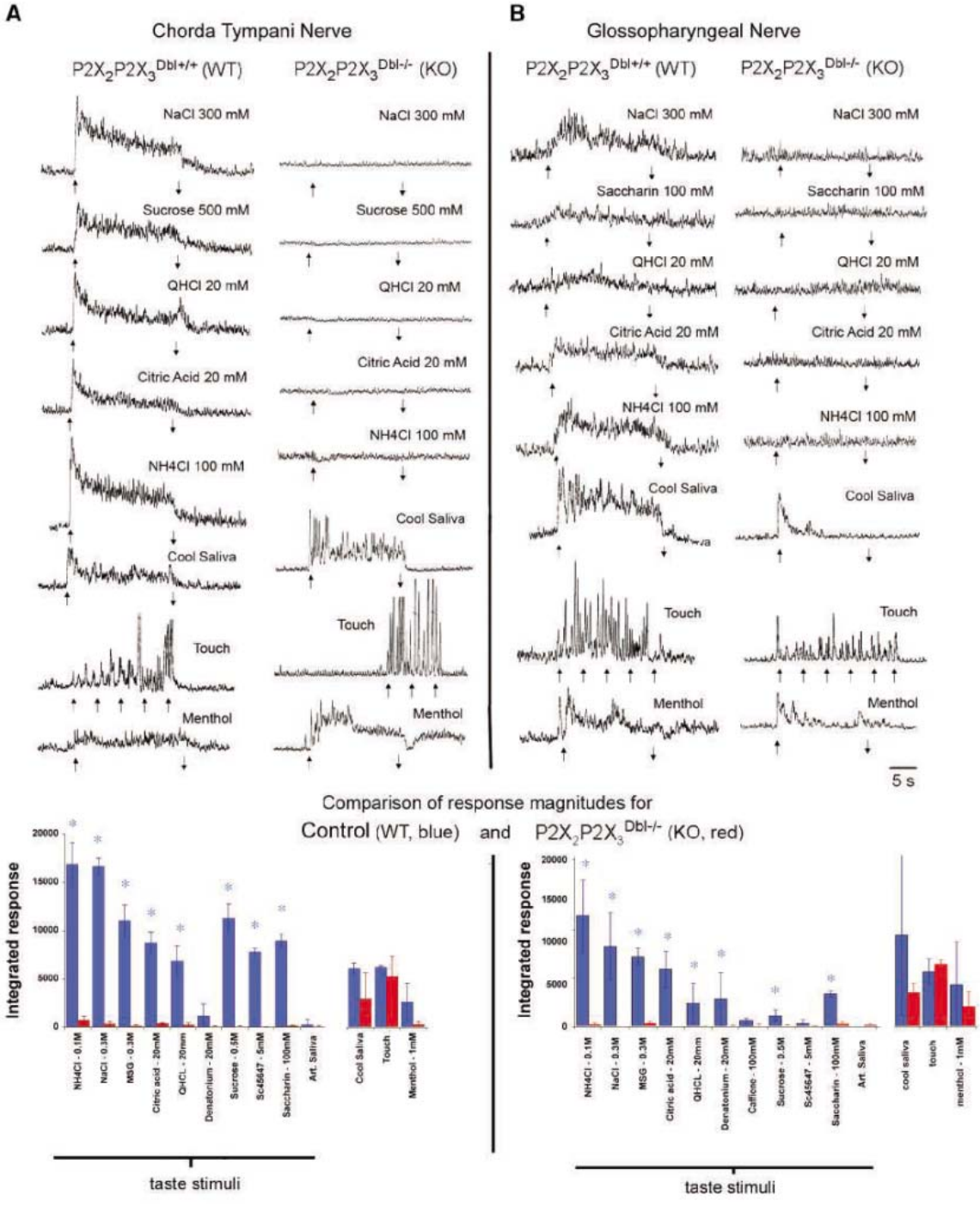

Fig. 1. (**A** and **B**) (Top) Gustatory nerve recordings from $P2X_2/P2X_3^{Dbl-/-}$ (KO) and $P2X_2/P2X_3^{Dbl/+/+}$ (WT) mice. (Bottom) Comparison of response magnitude of control mice (blue bars) and $P2X_2/P2X_3^{Dbl-/-}$ (KO) (red bars) to a variety of taste, tactile, and thermal stimuli. The responses of wild-type siblings do not differ significantly (ANOVA, $P > 0.01$) from responses recorded from C57BL/6J mice. The control group for the CT recordings includes data from both $P2X_2/P2X_3^{Dbl/+/+}$ (WT) and C57BL/6J mice. Blue asterisks indicate significant differences ($P < 0.05$) between control and KO groups (*t* test with Bonferroni correction for repeated measures).

not responsive to taste. These findings indicate that $P2X_2$ and/or $P2X_3$ receptors are essential for activation of the gustatory nerves.

To characterize taste-related behaviors of the $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice, two-bottle preference tests were used (*10*). Compared with $P2X_2/P2X_3^{Dbl/+/+}$(WT) mice, the $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice were not responsive to artificial sweeteners (Fig. 2, A and C), sucrose (Fig. 2B), or monosodium glutamate (Fig. 2D) and were largely unresponsive to many bitter substances including quinine hydrochloride (Fig. 2E) and denatonium benzoate (Fig. 2F). Single-KO mice, lacking either the $P2X_2$ or $P2X_3$ subunit, also were assessed in two-bottle preference tests for certain key tastants. The $P2X_3$-only KO animals were significantly impaired in their responses to SC45647, saccharin, and denatonium (fig. S2), whereas the $P2X_2$-only KO mice were only marginally impaired to denatonium. The single-KO strains were, however, at least an order of magnitude more responsive than the $P2X_2/P2X_3^{Dbl-/-}$ (KO) line. Thus, loss of either $P2X_2$ or $P2X_3$ alone resulted in only a moderate change in taste-mediated behaviors in contrast to the profound deficit seen in $P2X_2/P2X_3^{Dbl-/-}$ (KO) animals. This suggests that neither homomeric $P2X_2$ nor homomeric $P2X_3$ receptors suffice for normal function in this system.

The near-total loss of neural and behavioral responses to taste stimuli suggests a peripheral origin to the defect. We compared the morphology and innervation of the lingual taste buds in the $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice with $P2X_2/P2X_3^{Dbl/+/+}$ (WT) animals. As in $P2X_2/P2X_3^{Dbl/+/+}$ (WT) mice, the $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice showed a

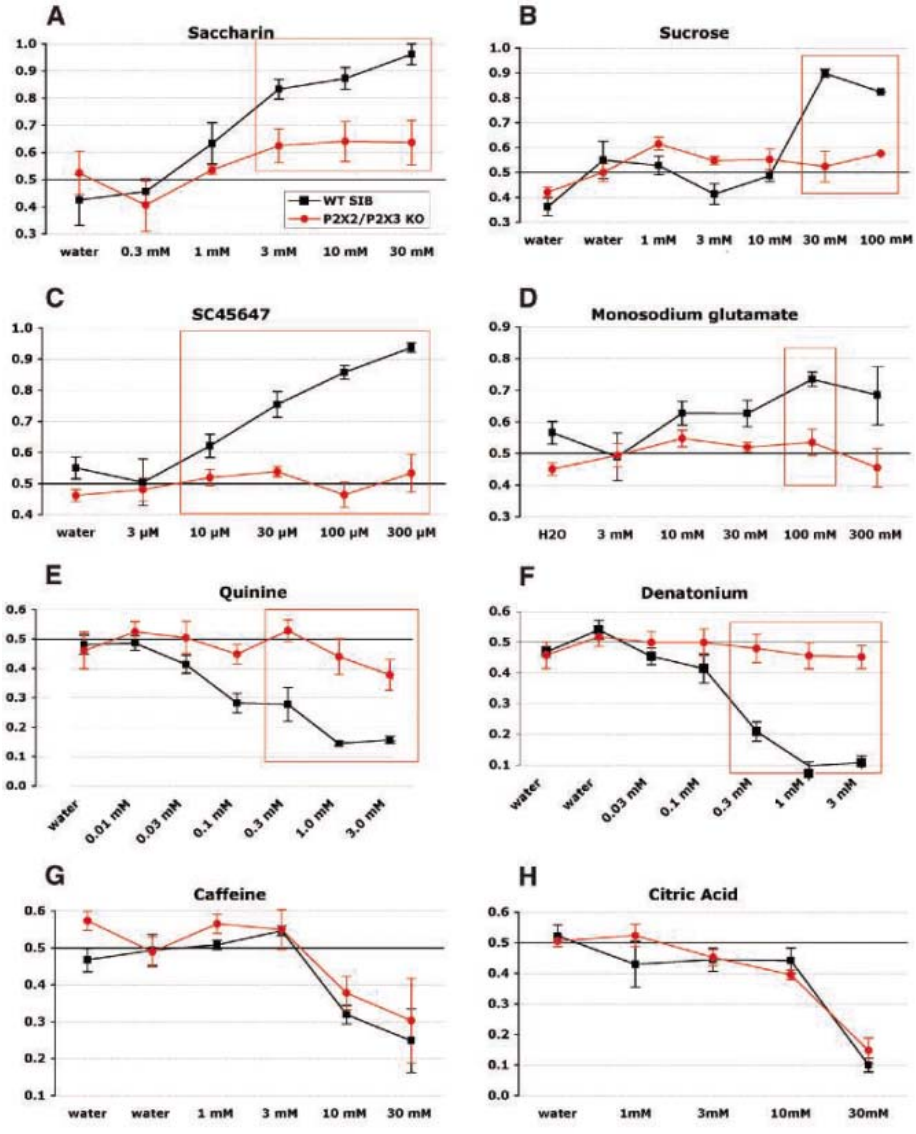

Fig. 2. (**A** to **H**) Behavioral data showing the $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice (red) do not respond to most taste substances and exhibit near-absent behavioral responses to other tastants including quinine and denatonium (bitter). For the artificial sweetener SC45647 (C), $P2X_2$-only KO mice are not significantly different from WT mice. In contrast, $P2X_3$-only KO mice are significantly impaired compared with WT but are significantly less impaired than the $P2X_2/P2X_3^{Dbl-/-}$ (KO) animals (fig. S2) (ANOVA, $P < 0.01$). Red boxes indicate points that are significantly different between $P2X_2/P2X_3^{Dbl-/-}$ (KO) and $P2X_2/P2X_3^{Dbl+/+}$ (WT) mice (*t* test with Bonferroni correction, $P < 0.05$).

normal complement of taste cells in fungiform, foliate, and circumvallate papillae (Fig. 3, A to D). Cells within the taste buds express the T1R taste receptors as assessed by in situ hybridization (Fig. 3, A to D for T1R1 and T1R2) and display roughly normal proportions of cells expressing gustducin, phospholipase C–β2 (PLC–β2), or serotonin, all markers of taste cells in normal mice. Thus, the peripheral taste apparatus appears intact both structurally and molecularly.

Despite the lack of neural response to any applied tastant in the $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice, the animals do exhibit near-normal avoidance to caffeine (bitter to humans) (Fig. 2G) and citric acid (sour) (Fig. 2H). These clear behavioral responses are unexpected, given the total lack of chorda tympani and glossopharyngeal gustatory nerve response to these same substances. Two possibilities may explain the behavioral results. Either these substances are being detected by chemoreceptors that are not taste buds, including those of the larynx, pharynx, esophagus, or gut, or nonlingual taste buds use different neurotransmitters than do lingual taste buds.

The chemical profile (acids and some bitter-tasting substances) of compounds detected by $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice is similar to the response profile of laryngeal chemoreceptors innervated by the superior laryngeal nerve (*11*, *12*). The larynx is replete with both taste buds and solitary chemoreceptor cells (SCCs) (*13*) lying within a specific laryngeal sensory epithelium (*14*). To determine whether either or both of these laryngeal chemoreceptors rely on purinergic neurotransmission, we used immunocytochemistry to localize $P2X_2$ and $P2X_3$ receptor subunits in transgenic mice in which green fluorescent protein (GFP) is expressed in gustducin-expressing taste cells and SCCs. Laryngeal taste buds were clearly innervated by nerve fibers immunoreactive for $P2X_2$ (Fig. 3F, red) and $P2X_3$ receptor subunits. In contrast, laryngeal SCCs, identified by gustducin-driven GFP expression, were not innervated by $P2X_2$ (Fig. 3G, compare Fig. 3E) or $P2X_3$ subunit–expressing nerve fibers. These results suggest that laryngeal taste buds may utilize purinergic neurotransmission, whereas laryngeal SCCs do not.

In order to assess whether avoidance of sour and bitter tastants in $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice is mediated by the gustatory system, we relied on tastant-induced expression of c-Fos within the brainstem. Activation of the gustatory system by strong tastants quickly activates expression of immediate early genes such as c-*fos* within the primary taste nucleus (NTS; nucleus of the solitary tract) in the brainstem (*15*, *16*). The lingual gustatory nerves terminate within the rostral and intermediate parts of the NTS, so presentation of tastants to the oral cavity evokes prominent c-Fos expression within the rostral and/or intermediate region of the NTS in both wild-type mice and rats. In contrast, in $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice, quinine (but not water) evokes little c-Fos activation in rostral or intermediate portions of NTS, but does evoke significant c-Fos expression in more caudal portions of the nucleus, where the superior laryngeal nerve and general visceral branches of the vagus nerve terminate (Fig. 3, H and I). These findings suggest that the behavioral avoidance seen in $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice is mediated by the superior laryngeal nerve or general visceral branch of the vagus conveying signals to the caudal NTS.

Finally, to test for tastant-evoked release of ATP, we used a standard luciferin-luciferase bioluminescence assay to detect ATP release from stripped epithelial preparations. For these experiments, taste bud–bearing epithelia of C57BL/6J mice were stripped from the vallate and foliate taste fields, as well as from nongustatory epithelium devoid of taste buds. Stimulation of the apical surface with buffer resulted in a basal level of ATP release as measured by the luminometer. When a mixture of two bitter compounds was applied to the apical surface of taste bud–bearing epithelium, the luminous flux was significantly greater, which indicated an increase in ATP concen-

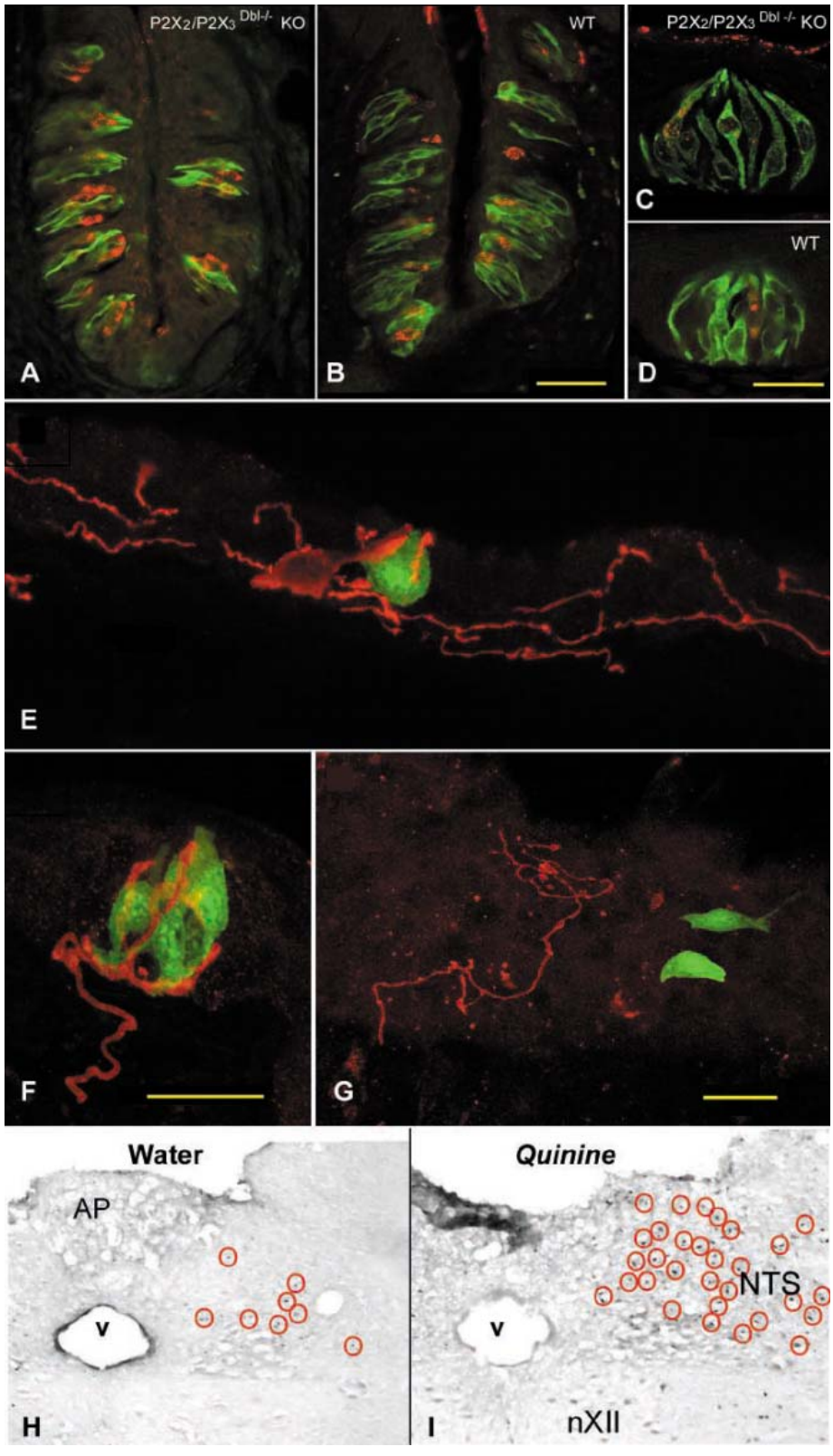

Fig. 3. Taste buds in $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice (**A** and **C**) are normal in terms of cell morphology and histochemistry compared with wild-type mice (**B** and **D**). (A and B) Taste buds in circumvallate papillae showing expression of gustducin (green) and T1R2 (red). (C and D) Palatal taste buds showing expression of gustducin (green) and T1R1 (red). (**E**) Laryngeal solitary chemoreceptor cells are densely innervated in wild-type mice. Gustducin immunoreactivity (green) in a solitary chemoreceptor cell showing dense innervation as revealed by immunoreactivity with the pan-neuronal marker PGP9.5 (red). (**F**) Laryngeal taste buds are innervated by purinoceptive nerve fibers expressing $P2X_2$ (red). Gustducin-expressing taste cells are green. This suggests that laryngeal taste buds, like lingual taste buds, rely on ATP as a transmitter. (**G**) Laryngeal solitary chemoreceptor cells (green) in a WT mouse are not innervated by P2X-expressing nerve fibers (red), although such fibers do innervate nearby epithelium. This indicates that nerve fibers that innervate laryngeal SCCs utilize a different neurotransmitter and/or receptor system. Compare this image with (E). (**H** and **I**) c-Fos immunoreactivity in the laryngeal portion of the nucleus of the solitary tract in $P2X_2/P2X_3^{Dbl-/-}$ (KO) mice. Activation of c-Fos in numerous cells (dark spots indicated by red circles) of this area indicates that quinine stimulates the laryngeal nerve, which sends information to this caudal portion of the nucleus. AP, area postrema; NTS, nucleus of the solitary tract; nXII, hypoglossal nucleus; v, 4th ventricle.

tration (Fig. 4). In contrast, stimulation of nongustatory epithelium with the bitter mixture evoked little or no ATP release. These findings demonstrate that ATP is released from taste epithelium when it is exposed to appropriate taste stimuli.

Our results strongly suggest that ATP serves as a key neurotransmitter linking taste buds to sensory nerve fibers. Criteria for a neurotransmitter include release, the presence of specific receptors, and a mechanism for clearance. Our luminometer data demonstrate tastant-evoked release of ATP. The immunocytochemical studies by Bo *et al.* (*5*), as well as our own results, show the presence of postsynaptic P2X receptors, whereas the physiological and behavioral studies demonstrate that the P2X receptors are necessary for

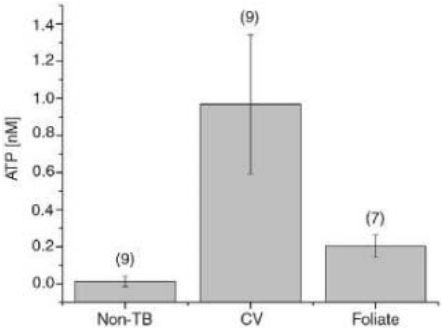

Fig. 4. Release of ATP from taste epithelium when stimulated with a bitter mixture containing denatonium and quinine. Nongustatory epithelium (non-TB) and taste bud–bearing epithelial sheets containing either circumvallate (CV) or foliate papillae were placed in an Ussing-type chamber that permits selective application of taste stimuli to the apical membrane. ATP released from the basolateral compartment was collected in the luciferase assay buffer and transferred to the luminometer for measurement of relative light units, which were converted into ATP concentration. Stimulation of taste epithelia with the bitter mixture significantly increases ATP release (mean ± SEM) from CV and foliate tissues relative to non-TB tissue ($P < 0.05$, *t* test).

sensory transmission in this system. Finally, extracellular ATP is rapidly degraded by ecto-ATPases known to be abundantly present in taste buds (*17*–*19*). Thus, ATP meets all the essential criteria for being the major neurotransmitter of the peripheral taste system. Other neuropeptides and transmitters observed within taste buds (*1*, *2*, *20*, *21*) likely play a modulatory role or may be crucial for intragemmal communication among the different types of taste cells.

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Materials and Methods
Figs. S1 and S2
References and Notes

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Chromosome Alignment and Segregation Regulated by Ubiquitination of Survivin

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Proper chromosome segregation requires the attachment of sister kinetochores to microtubules from opposite spindle poles to form bi-oriented chromosomes on the metaphase spindle. The chromosome passenger complex containing Survivin and the kinase Aurora B regulates this process from the centromeres. We report that a de-ubiquitinating enzyme, hFAM, regulates chromosome alignment and segregation by controlling both the dynamic association of Survivin with centromeres and the proper targeting of Survivin and Aurora B to centromeres. Survivin is ubiquitinated in mitosis through both Lys^{48} and Lys^{63} ubiquitin linkages. Lys^{63} de-ubiquitination mediated by hFAM is required for the dissociation of Survivin from centromeres, whereas Lys^{63} ubiquitination mediated by the ubiquitin binding protein Ufd1 is required for the association of Survivin with centromeres. Thus, ubiquitinaton regulates dynamic protein-protein interactions and chromosome segregation independently of protein degradation.

Mitosis is one of the visually most dynamic cellular processes, requiring a continuous assembly and disassembly of many protein complexes. We studied the chromosome passenger protein Survivin, which exhibits a dynamic interaction with centromeres (*1*–*3*). To identify proteins that interact with Survivin and thus may regulate the dynamic binding of Survivin to centromeres, we raised rabbit polyclonal antibodies to *Xenopus* Survivin (*4*). The antibody immunoprecipitated Survivin and seven other proteins from *Xenopus* egg extracts (p1 to p7) (fig. S1A). Western blotting revealed that two components of the chromosome passenger complex, inner centromere protein (INCENP) and the protein kinase Aurora B, were immunoprecipitated by the Survivin antibody.

We micro-sequenced the proteins corresponding to p1, p4, p5, and p7 (fig. S1A). Protein p1 is a homolog of the human protein USP9x (an X-linked ubiquitin specific protease, Genbank accession number NP004643) (*5*, *6*) and a mouse protein, FAM (fat facet in mouse, accession number P70398) (*7*), which share ~98% amino acid identity with one another. FAM and USP9x are homologs of the *Drosophila* protein faf (fat facet), which is required for cellularization in early embryos and for cell-fate determination in the *Drosophila* eye (*8*). Little is known about the function of USP9x, but both faf and FAM function as deubiquitinating (Dub) enzymes and can regulate protein trafficking (*9*–*14*). We refer to the *Xenopus* protein as xFAM and the human USP9x as hFAM. The proteins corresponding to p4, p5, and p7 were identified as the p97 adenosine triphosphatase associated with various cellular activities (AAA ATPase), the nuclear protein localization 4 (Npl4), and the ubiquitin fusion degradation 1 (Ufd1), respectively. The p97 protein forms a homohexameric ring that interacts with the Npl4-Ufd1 heterodimer. The resulting complex functions as a ubiquitin-selective chaperone to regulate protein ubiquitination and degradation (*15*, *16*).

Because all four proteins that coimmunoprecipitated with Survivin are involved in the ubiquitin-mediated signaling, we reasoned that

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Survivin function in mitosis might be regulated by ubiquitination. To test this idea, we focused on the de-ubiquitinating enzyme hFAM. We generated five antibodies to hFAM, with four peptides and a fusion protein as antigens (fig. S1B) (*4*). All five antibodies recognized hFAM and xFAM by Western blotting and by immunoprecipitation (fig. S1, C and D). Reciprocal immunoprecipitation confirmed that xFAM and *Xenopus* Survivin interacted with one another in *Xenopus* egg extracts (fig. S1E). Furthermore, Myc-tagged human Survivin immunoprecipitated with hFAM from HeLa cells (fig. S1F). None of our affinity-purified hFAM antibodies revealed specific localization of hFAM in HeLa cells or mouse NIH 3T3 cells, suggesting that FAM is evenly distributed in these cells.

We inhibited the expression of hFAM in HeLa cells with two small interfering RNAs (siRNAs) (hFAM-s1 or hFAM-s2). A Survivin siRNA (*17*) and luciferase siRNA were used as controls (*18*). Western blotting revealed that siRNAs of hFAM and Survivin decreased the expression of proteins, whereas the control luciferase siRNA had no effect on either protein (Fig. 1A). Decreased expression of either hFAM or Survivin inhibited cell proliferation (Fig. 1B). However, decreased expression of hFAM did not affect the expression of Survivin or vice versa (Fig. 1A). Thus, hFAM appears not to regulate the stability of Survivin. Decreased expression of either Survivin or hFAM resulted in an increase in misaligned chromosomes in metaphase and lagging chromosomes in anaphase (Fig. 1C). (We defined misaligned chromosomes in metaphase as chromosomes that failed to align with the majority of chromosomes at metaphase plates.) Furthermore, 4′,6′-diamidino-2-phenylindole (DAPI) staining revealed that down-regulation of hFAM or Survivin led to an increase of binucleated or multinucleated cells from ~5% in control to ~12% in hFAM RNA interference (RNAi)–treated cells and ~50% in Survivin RNAi-treated cells (Fig. 1D), suggesting that hFAM has a minor role, if any, in cytokinesis, compared with that of Survivin. Decreased expression of FAM in NIH 3T3 cells with hFAM-s1 treatment (hFAM-s1 sequence is identical in mouse) also caused similar defects in cell division.

The cell-division defects observed after inhibition of hFAM expression were largely rescued by the expression of a FAM molecule mutagenized at three wobble codons, resulting in a FAM gene that encodes wild-type (WT) protein ($pFAM^{INS}$) and is insensitive to the siRNA. But, the defects were not fixed by expressing the nonmutagenized FAM^{WT} (*4*) (Fig. 1E and fig. S1G). Thus, hFAM, like Survivin, regulates chromosome alignment and segregation in mitosis.

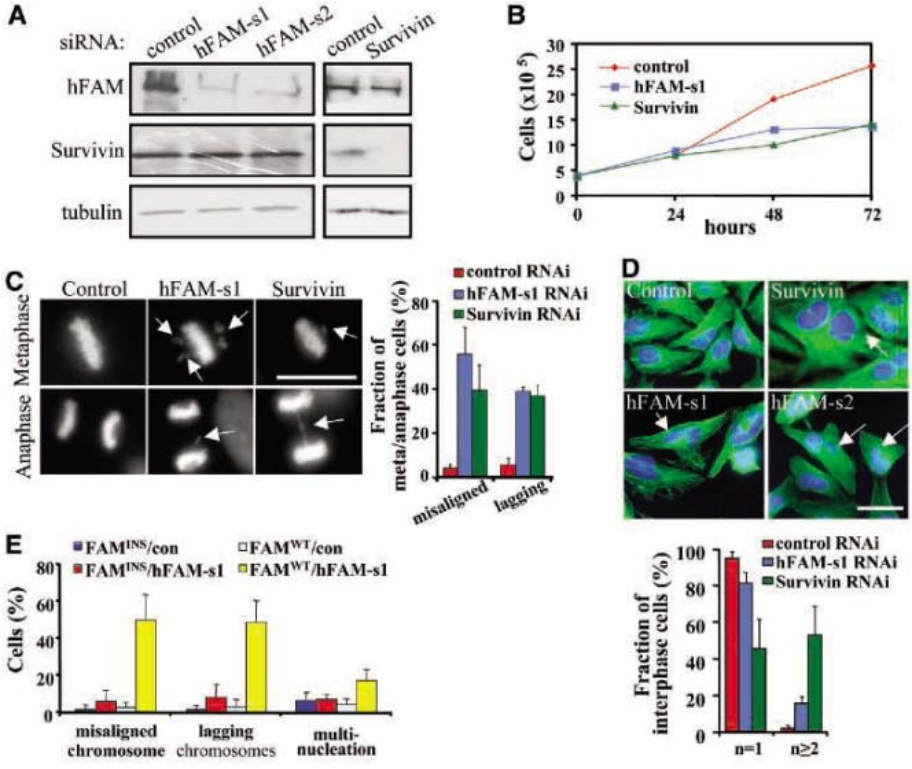

Fig. 1. hFAM characterization. (**A**) Western blotting of HeLa cells treated with siRNAs corresponding to luciferase (control), hFAM (hFAM-s1 or hFAM-s2), and Survivin. Survivin and hFAM were down-regulated ~80% by their respective siRNAs. Western blotting of tubulin served as loading controls. (**B**) Down-regulation of hFAM or Survivin by their respective siRNAs reduced cell proliferation as compared to the control siRNA treatment. (**C**) Accumulation of misaligned and lagging chromosomes in metaphase and anaphase after depletion of hFAM or Survivin, respectively. HeLa cells treated with the indicated siRNA were stained by DAPI (left). The percentages of mitotic cells with misaligned or lagging chromosomes in metaphase or anaphase, respectively, were quantified (right). (**D**) Accumulation of bi-nucleated or multinucleated cells after depletion of hFAM or Survivin. HeLa cells (top) treated with the indicated siRNA were stained with antibody to tubulin (green) and DAPI (blue). Arrows point to cells with more than one nucleus. The percentages of interphase cells with single ($n = 1$) or more ($n \geq 2$) nuclei were quantified (bottom). (**E**) Cell-division defects were caused by depletion of hFAM. Expression of FAM^{INS} significantly reduced the defects caused by hFAM RNAi-treatment. Error bars indicate standard deviation (SD) from at least three independent experiments. For chromosome segregation defects, 100 mitotic cells were analyzed for each RNAi experiment. Scale bars in (C) and (D), 20 μm.

We tested whether hFAM regulates Survivin binding to centromeres in mitosis. Centromeres were detected using the anti-centromere antibody (ACA) that recognizes several centromere proteins (*19*), and Survivin was detected with Survivin antibodies. ACA staining of centromeres appeared as bright dots in both control and hFAM RNAi-treated cells (Fig. 2, A and B) (*20*). However, although the overall Survivin levels on the prometaphase chromosomes or metaphase-aligned chromosomes were similar in control or hFAM RNAi-treated cells, Survivin staining of centromeres appeared more diffuse in hFAM RNAi-treated cells (Fig. 2, A and B).

In prometaphase control cells, most focused ACA-stained dots corresponded to strongly focused Survivin dots. In contrast, in cells treated with hFAM siRNA, many focused ACA dots did not correspond to focused Survivin dots (Fig. 2, A and C). In metaphase control cells, Survivin dots were flanked by pairs of ACA dots on centromeres of chromosomes aligned at the metaphase plate. However, in hFAM RNAi-treated cells, the majority of focused ACA dots did not flank Survivin dots (Fig. 2, B and C). Double immunostaining with antibodies to ACA and Aurora B revealed similar defects of Aurora B localization in hFAM RNAi-treated cells. Thus, the depletion of hFAM disrupted normal localization of Survivin, which could lead to chromosome misalignment and segregation.

We also examined Survivin localization on the misaligned chromosomes. Survivin staining on these chromosomes was brighter at the centromeres and appeared to spread into chromosome arms in hFAM RNAi-treated cells, compared with the staining on rare misaligned chromosomes in controls (Fig. 2D). Quantification revealed an approximate twofold increase in total Survivin stain-

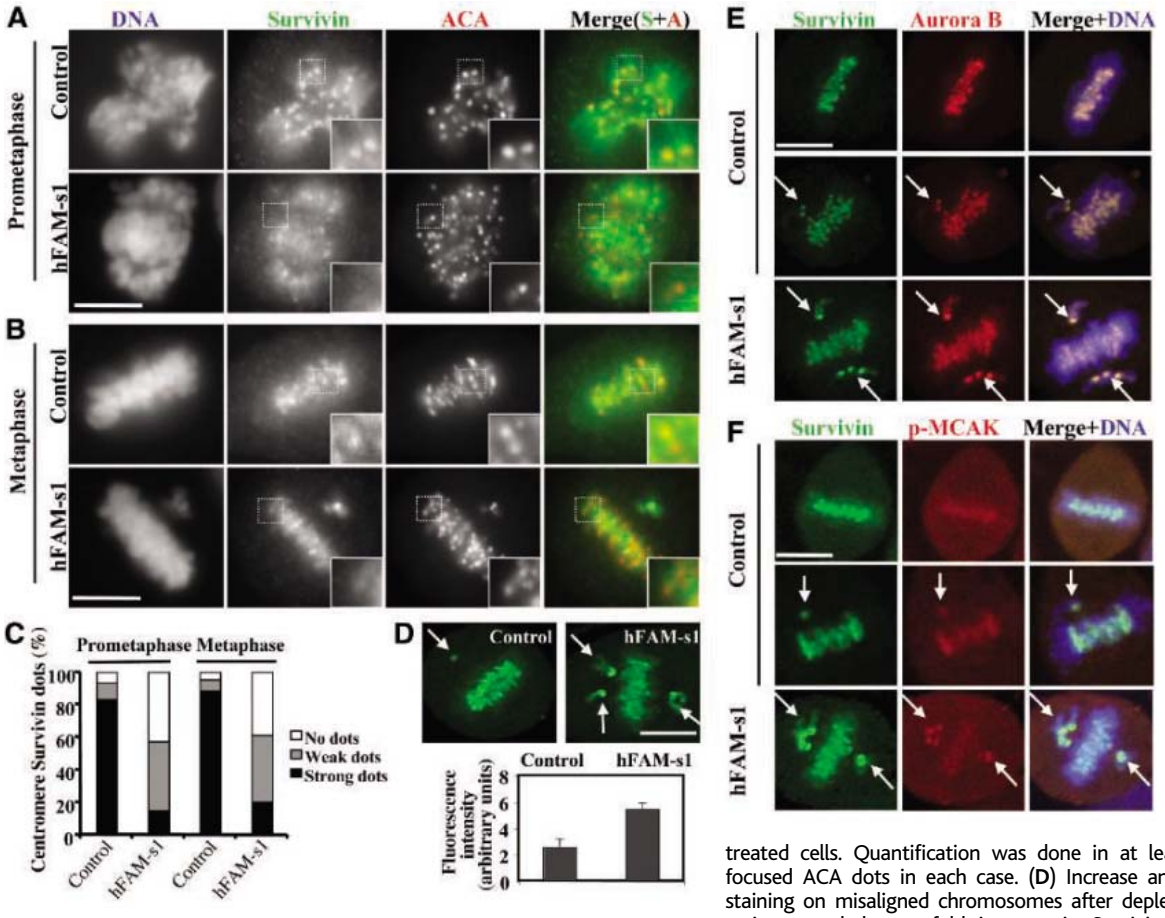

Fig. 2. Regulation by hFAM of the localization of Survivin and Aurora B to centromeres in mitosis. (**A** and **B**) Survivin in prometaphase (A) and metaphase (B) cells treated with or without hFAM-s1. HeLa cells were stained by DAPI (DNA) and by antibodies to Survivin (green) and ACA (red). The images were taken by focusing on ACA-positive dots. Focused ACA dots and their corresponding areas in the Survivin-staining channel are highlighted by squares and enlarged in the right corners of each image. (**C**) Reduced number of centromeres with focused Survivin staining after depletion of hFAM. Percentages of centromeres (identified as focused ACA dots) having no, weak, or strong dots of Survivin were quantified in control and hFAM RNAi-treated cells. Quantification was done in at least 10 cells with ~100 focused ACA dots in each case. (**D**) Increase and expansion of Survivin staining on misaligned chromosomes after depletion of hFAM. Quantification revealed ~twofold increase in Survivin staining on misaligned chromosomes in hFAM RNAi-treated cells, compared with controls. Error bars indicate SD from >42 cells. (**E**) Increased and expanded staining of Aurora B on centromeres of misaligned chromosomes after hFAM depletion. (**F**) Aurora B phosphorylation of MCAK (p-MCAK) at the centromeres of misaligned chromosomes after hFAM depletion. Arrows in panels point to misaligned chromosomes. Scale bars, 10 μm.

ing on the misaligned chromosomes in hFAM RNAi-treated cells, compared with that in control cells (Fig. 2D). Aurora B staining was also expanded beyond centromeres on the misaligned chromosomes in hFAM RNAi-treated cells (Fig. 2E).

Phosphorylation of the mitotic centromere-associated kinesin (MCAK) by Aurora B inhibits MCAK's ability to depolymerize microtubules at the kinetochore (*21*, *22*). The balance between phosphorylation of MCAK by Aurora B and dephosphorylation by phosphatase I at kinetochores is thought to allow proper microtubule attachment to kinetochores. Thus, excess accumulation of Survivin and Aurora B on misaligned chromosomes of hFAM RNAi-treated cells might increase MCAK phosphorylation at kinetochores, which might prevent MCAK from correcting chromosome misalignment. Indeed, immunostaining with an antibody that recognizes Aurora B–phosphorylated MCAK (*21*) showed an increased accumulation of phospho-MCAK on misaligned centromeres in hFAM RNAi-treated cells compared with those in controls (Fig. 2F and fig. S2). Thus, hFAM regulates Survivin, which in turn regulates MCAK phosphorylation by Aurora B at the centromeres.

We found no obvious difference in the localization of Survivin and Aurora B to central spindles in anaphase and midbodies in telophase in hFAM RNAi-treated cells (fig. S3), suggesting that hFAM specifically regulates the chromosome-segregation function, but not the cytokinesis function, of Survivin. The bi-nucleation or multinucleation in hFAM RNAi-treated cells (Fig. 1D) may result from lagging chromosomes that block the closure of cytokinesis furrows.

We used both fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) to measure the binding kinetics of Survivin to centromeres in mitosis (*23*). Control treatment or hFAM RNAi treatment was applied to HeLa cells that were transiently expressing Survivin–green fluorescent protein (GFP) (*24*). Expression of Survivin-GFP can rescue HeLa cells from Survivin RNAi (*25*), so we presumed that Survivin-GFP could be used to report the behavior of endogenous Survivin (fig. S4). Using FRAP, we found that in control RNAi cells, the half-time ($t_{1/2}$) of Survivin-GFP recovery was 2 to 6 s (*3*). However, the $t_{1/2}$ for Survivin-GFP recovery in hFAM RNAi-treated cells was increased to 80 to 100 s on prometaphase and metaphase chromosomes (Fig. 3, A and B, and fig. S5) (*26*). There was also an increase in the immobile fraction of Survivin on the misaligned centromeres (table S3). Survivin-GFP on misaligned metaphase chromosomes had a slower recovery than that on aligned chromosomes in hFAM RNAi-treated cells (Fig. 3B). Using FLIP, we found that Survivin-GFP dissociated from centromeres faster in control RNAi cells than in hFAM RNAi-treated cells in both prometaphase and metaphase (Fig. 3 C and D, and fig. S5) (*26*). Furthermore, in hFAM RNAi-treated cells, Survivin-GFP on the misaligned chromosomes dissociated more slowly than that on aligned chromosomes (Fig. 3C and table S3). This slower dissociation is consistent with the observation of an increased accumulation of Survivin on misaligned chromosomes in hFAM RNAi-treated cells (Fig. 2D). Thus, hFAM appears to control centromeric localization of Survivin by regulating the dynamic dissociation of Survivin from centromeres. Survivin-GFP at central spindles

and midbodies exhibited similar FRAP behavior in control and in hFAM RNAi-treated cells (fig. S6), consistent with the idea that hFAM does not regulate the cytokinesis function of Survivin.

Because polyubiquitination through Lys^{63} (K63) of ubiquitin regulates protein-protein interactions but not protein degradation (*27*), we tested whether hFAM might regulate the dynamic interaction of Survivin with centromeres by controlling the level of K63 ubiquitination on Survivin. We characterized Survivin ubiquitination in mitosis in HeLa cells transfected with Myc-tagged Survivin, wild-type hemagglutinin (HA)–tagged ubiquitin, or mutant HA-tagged ubiquitins that mediate only K48 or K63 linkages. Myc-tagged Survivin was analyzed by immunoprecipitation and Western blotting (*28*). Survivin was ubiquitinated through K63 and K48 linkages in mitosis, and the ubiquitination was not caused by mitotic arrest (fig. S7, A and B). Furthermore, the expression of a FAM fragment possessing the Dub catalytic domain and Dub activity ($V5FAM^{CAT}$, fig. S1B) (*13*, *14*) reduced the wild-type (contains both K63 and K48 linkages) and the K63-linked ubiquitin on Survivin to about half of the controls. However, the K48-linked ubiquitination was not affected (Fig. 4A and fig. S7C). The modest reduction of ubiquitination reflects the modest expression of $V5FAM^{CAT}$ (fig. S7C). We were unable to overexpress either FAM^{CAT} or full length FAM in cells.

The expression of $pV5FAM^{CAT}$ in cells treated with hFAM-s1 (hFAM-s1 is upstream of the $V5FAM^{CAT}$ fragment, fig. S1B) led to a reduction of misaligned and lagging chromosomes and of bi-nucleated or multi-nucleated cells (Fig. 4B). Thus hFAM RNAi appears to increase the amount of K63-linked ubiquitination on Survivin, which disrupts accurate targeting of Survivin to centromeres, leading to chromosome misalignment and missegregation in mitosis.

Because hFAM RNAi caused cell death, we were unable to assay K63 ubiquitination on Survivin after exposing cells to hFAM RNAi. Therefore, we sought to study the effect of K63 ubiquitination on Survivin by mutagenizing the lysines (K) to arginines (R) on Survivin. K residues in proteins are involved in a number of posttranslational modifications including ubiquitination, sumoylation, methylation, and acetylation. Because posttranslational modifications of different K residues in a protein could regulate one another (*29*), it may be possible to create Survivin mutants that have increased or decreased K63 ubiquitination. There are 16 K residues in human Survivin. Nine of these (K23, K62, K78, K79, K90, K91, K110, K112, and K115) are highly conserved. On the basis of the crystal structure of Survivin, four of these nine Ks (K23, K62, K78, and K79) are clustered in the N terminus of Survivin that forms the Bir [baculovirus Ile-Ala-Pro (IAP) repeat] domain, whereas the remaining five Ks (K90, K91, K110, K112, and K115) are clustered in the C terminus (*30*).

We mutagenized all 16 Ks into Rs, but this mutant Survivin was not expressed in cells. Even when only the nine conserved Ks were changed to Rs, the mutant Survivin expression level was still too low to allow determination of its localization in cells. Survivin mutant (Sur4K-R) with four Ks (K23, K62, K78, and K79) changed to Rs was expressed and bound to centromeres (Fig. 4C). However, not every centromere (identified by ACA) had clear Sur4K-R localization (fig. S8, A and B). Ubiquitination assays revealed that, compared with wild-type Survivin, cells expressing Sur4K-R had increased ubiquitination that was mostly accounted for by the K63 linkage (Fig. 4D).

We also used FRAP to study the interaction between Sur4K-R and centromeres. Sur4K-R-GFP or wild-type Survivin (Sur-GFP) was transfected into HeLa cells for FRAP analysis. Sur4K-R-GFP showed reduced FRAP compared with that of Sur-GFP

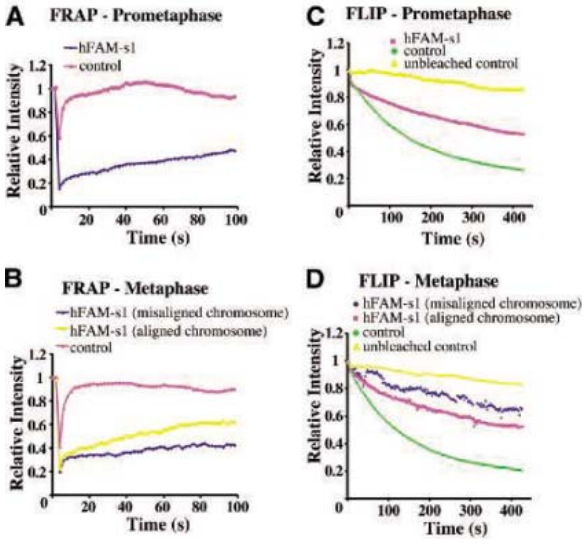

Fig. 3. Regulation by hFAM of the dynamic turnover of Survivin on mitotic centromeres. (**A** and **B**) FRAP analyses of Survivin-GFP in prometaphase (A) and metaphase (B) cells treated with either hFAM-s1 or control siRNA. (**C** and **D**) FLIP analyses of Survivin-GFP in prometaphase (C) and metaphase (D) cells. FLIP was quantified by plotting the loss of Survivin-GFP on all prometaphase chromosomes or aligned metaphase chromosomes over time in both control and hFAM RNAi-treated cells. FLIP on the misaligned chromosomes in metaphase was also quantified in hFAM RNAi-treated cells. The FLIP and FRAP curves show averages of at least six independent experiments.

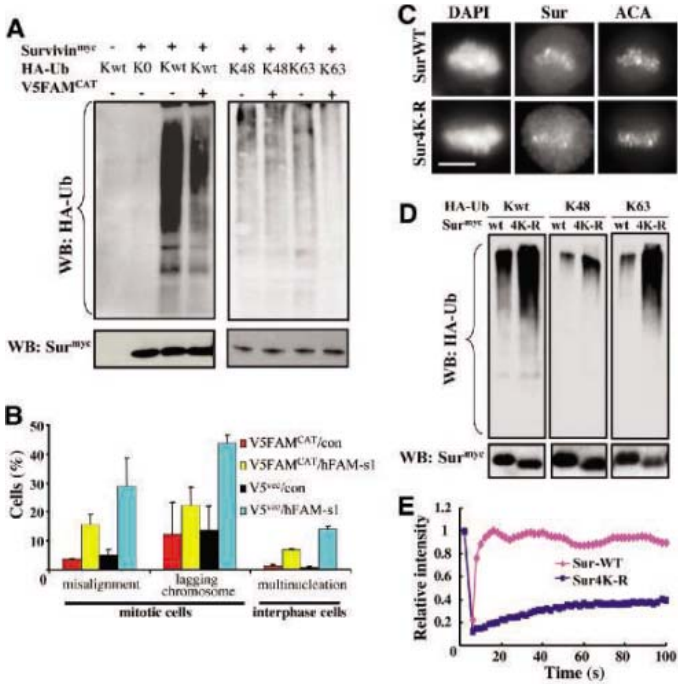

Fig. 4. Deubiquitination of Survivin by hFAM. (**A**) Effect of $V5FAM^{CAT}$ on wild-type, K63-, or K48-ubiquitination of Survivin. (**B**) Effect of $V5FAM^{CAT}$ on cell-division defects caused by hFAM-s1. Vector control ($pV5^{vec}$) or $pV5FAM^{CAT}$ were used to transfect HeLa cells treated with control siRNA (con) or hFAM-s1. Error bars indicate SD from at least three independent experiments. (**C**) Localization of Survivin. Mutant Survivin ($Sur4K\text{-}R^{Myc}$) or wild-type $Survivin^{Myc}$ were transfected into HeLa cells. Survivin, centromeres, or DNA were detected using antibody to either Myc or ACA, or DAPI, respectively. (**D**) Ubiquitination assay of wild-type Survivin and Sur4K-R. (**E**) FRAP. Sur4K-R was subcloned into pEGFP-N3 to make Sur4K-R-GFP. FRAP analyses were carried out in cells expressing either Sur4K-R-GFP or wild-type Sur-GFP. The FRAP curves show averages from at least six different cells.

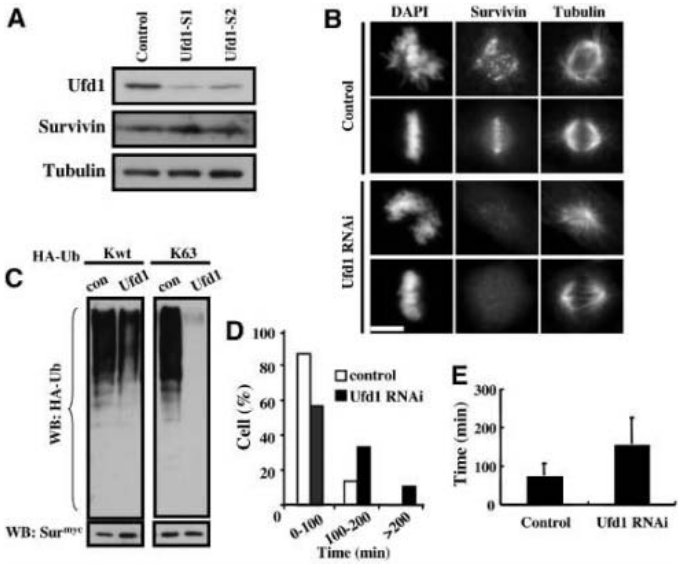

Fig. 5. Regulation of Survivin by Ufd1. (**A**) Depletion of Ufd1 by two different siRNAs (Ufd1-S1 or Ufd1-S2) did not change Survivin protein expression level. Tubulin served as loading controls. (**B**) Ufd1-regulated binding of Survivin to centromeres. Control or Ufd1 RNAi-treated cells were processed for immunofluorescence to detect DNA, Survivin, and microtubules. Representative prometaphase and metaphase cells are shown. (**C**) Ufd1 regulates K63 ubiquitination of Survivin. Survivin ubiquitination by either wild-type ubiquitin (Kwt) or mutant ubiquitin (K63) was analyzed in cells treated with either control (con) or Ufd1 RNAi. (**D**) Regulation of mitotic progression by Ufd1. Cells were treated with control or Ufd1 siRNA for 48 hours followed by imaging on a temperature-controlled stage at 3-min intervals for 12 to 16 hours using a Hoffman modulation contrast objective lens (10×) on a Nikon TE200 microscope equipped with an Orca-2 camera. The graph shows quantification of elapsed time for cells that progressed from round-up to chromosome separation. At least 50 mitotic cells were analyzed in either control or Ufd1 RNAi-treatment. (**E**) Chromosome alignment regulated by Ufd1. HeLa cells stably expressing GFP-H2B were treated with control or Ufd1 siRNA for 48 hours followed by imaging at 3-min intervals for 12 to 16 hours using a fluorescence objective lens (20×). The graph shows quantification of time elapsed in prometaphase and metaphase in control or Ufd1 RNAi-treated cells. Control cells spent shorter time to achieve metaphase chromosome alignment than did Ufd1 RNAi-treated cells (*t* test, $P < 0.01$). Error bars indicate SD. Scale bar, 10 μm.

(Fig. 4E and fig. S8C). The FRAP kinetics of Sur4K-R-GFP (Fig. 4E) were similar to those of wild-type Sur-GFP in hFAM RNAi-treated cells (Fig. 3, A and B).

The above studies indicate that excessive K63 ubiquitination of Survivin blocks its dissociation from centromeres. Thus, insufficient K63 ubiquitination might inhibit the binding of Survivin to centromeres. The p97-Ufd1-Npl4 complex, which co-immunoprecipitated with Survivin and FAM (fig. S1A), recruits ubiquitin ligase to substrates to extend the ubiquitin chains on the substrates (*16*). This complex regulates spindle disassembly at the end of mitosis (*15*), and we reasoned that it might have an earlier mitotic role by promoting K63 ubiquitination of Survivin and chromosome alignment.

We verified the interaction between Survivin and the complex in HeLa cells expressing V5-tagged Ufd1 and Myc-tagged Survivin (fig. S9A). Then we used siRNAs directed at different regions of Ufd1 (Ufd1-S1 or Ufd1-S2) to disrupt the function of the p97-Ufd1-Npl4 complex (*18*). The depletion of Ufd1 by either siRNA did not affect Survivin protein levels (Fig. 5A).

Both siRNAs had similar effects. Decreased expression of Ufd1 did not block bipolar spindle assembly (Fig. 5B) (*15*), but it consistently reduced the K63 ubiquitination of Survivin (Fig. 5C). The partial reduction of the total ubiquitination of Survivin, as assayed with wild-type ubiquitin, can be accounted for by the reduction in K63-linked ubiquitination (Fig. 5C). The effect of Ufd1 down-regulation on K48 ubiquitination of Survivin was variable, ranging from no effect to mild reduction. Thus, Ufd1 appears to be required primarily for K63 ubiquitination of Survivin in mitosis.

Immunofluorescence microscopy revealed that Survivin staining of centromeres was either absent or reduced in cells treated with Ufd1 RNAi (Fig. 5B). We stained the centromeres with Survivin and ACA antibodies. Ufd1 RNAi did not affect ACA staining of centromeres (fig. S9B). Whereas over 80% of centromeres had strong Survivin staining in control RNAi-treated cells, less than 10% of centromeres had strong Survivin staining in Ufd1 RNAi-treated cells (fig. S9C). Aurora B staining of centromeres was also absent or reduced in the Ufd1 RNAi-treated cells (fig. S9D). Thus, K63 ubiquitination of Survivin appears to be required for centromere targeting.

We imaged cell division in live control or Ufd1 RNAi-treated cells by Hoffman modulation contrast. Metaphase chromosomes appear as a distinctive bar at the middle of the cell, and the metaphase-anaphase transition can be clearly detected when the metaphase chromosomal bar splits into two (fig. S9E). We quantified the time elapsed from the beginning of mitosis (judged by cell round-up) to the time when the metaphase chromosome bar separated into two in control and Ufd1 RNAi-treated cells. The Ufd1 RNAi-treated cells took a longer time to complete chromosome segregation (Fig. 5D). This observation suggests that Ufd1 RNAi-treated cells have difficulty in achieving chromosome alignment in mitosis.

To further determine whether the lack of Survivin at centromeres affected chromosome alignment, we imaged chromosomes in HeLa cells expressing GFP-histone H2B. Many Ufd1 RNAi-treated cells took longer to achieve metaphase chromosome alignment (fig. S9F). Quantification revealed that the time cells spent in prometaphase and metaphase was significantly longer in the Ufd1 RNAi-treated cells than in control cells (Fig. 5E). Thus, Survivin ubiquitination on K63 is required for its centromere targeting and chromosome alignment in mitosis.

Ubiquitin has a well-established role in targeting proteins for degradation. However, it also regulates DNA repair (*31*) and nuclear factor κB (NF-κB) signaling (*32*) in a protein degradation–independent manner. Our studies reveal that the protein degradation–independent signaling of ubiquitination is important in regulating dynamic protein targeting in mitosis. Degradation of Survivin by proteasomes is controlled by K48-linked ubiquitination at the end of mitosis (*33*). We propose that a balanced K63-linked ubiquitination and deubiquitination of Survivin is necessary for the correct targeting of Survivin and other chromosome passenger proteins to centromeres in mitosis. This in turn regulates a balanced phosphorylation and dephosphorylation of MCAK and chromosome alignment. Many ubiquitin ligases, Dubs, and their regulators have been identified in eukaryotic genomes (*34*, *35*). These proteins may have far-reaching roles in regulating dynamic protein-protein interactions in mitosis that are independent of protein degradation.

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was raised against glutathione S-transferase (GST) *Xenopus* Survivin and affinity purified using cleaved Survivin. Antibodies against hFAM were raised against each of four peptides (TPPDEQGQGDAPPQLED, CAPDEHESPPPEDAP, QRAQENYEGSEEVSPPQTKDQ, and GDEKQDNESNVDPRDDV) (*36*) and one GST fusion of the C-terminal fragment (amino acids 2347 to 2547) of hFAM. All were affinity purified against the respective antigens. Mouse Ufd1 was cloned into the pEF6/V5-His vector. Ufd1 antibodies were raised against GST-Ufd1 fusion protein and purified against His-tagged Ufd1. Antibodies against tubulin (Sigma), ACA (Antibodies Incorporated), human Survivin (R&D systems), human Aurora B (BD Biosciences), HA (Roche), Myc (Santa Cruz), and control immunoglobulin G (IgG) (Jackson Laboratory) were purchased. Antibodies against phosphorylated MCAK were described previously (*21*). Immunoprecipitation and immunofluorescence were carried out as described previously (*15*).

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37. We thank J. Swedlow, S. Wood, H. Meyer, and T. Dawson for antibodies and constructs; B. Lane for protein sequencing; C. Pickart for helpful advice on ubiquitination assays; O. Martin and R. Chen for excellent technical support; and M. Guo, D. Koshland, J. Yanowitz, and the members of Zheng lab for helpful comments. This work was supported by Howard Hughes Medical Institution and by National Institute of General Medical Sciences grant no. GM56312.

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5753/1499/DC1
Materials and Methods
Figs. S1 to S11
Tables S1 to S3
References

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Prostaglandin E_2 Promotes Colon Cancer Cell Growth Through a G_s-Axin-β-Catenin Signaling Axis

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How cyclooxygenase-2 (COX-2) and its proinflammatory metabolite prostaglandin E2 (PGE2) enhance colon cancer progression remains poorly understood. We show that PGE2 stimulates colon cancer cell growth through its heterotrimeric guanine nucleotide-binding protein (G protein)–coupled receptor, EP2, by a signaling route that involves the activation of phosphoinositide 3-kinase and the protein kinase Akt by free G protein βγ subunits and the direct association of the G protein α_s subunit with the regulator of G protein signaling (RGS) domain of axin. This leads to the inactivation and release of glycogen synthase kinase 3β from its complex with axin, thereby relieving the inhibitory phosphorylation of β-catenin and activating its signaling pathway. These findings may provide a molecular framework for the future evaluation of chemopreventive strategies for colorectal cancer.

Colorectal cancer represents the third leading cause of cancer-related deaths in both men and women in the United States (*1*). The development of colon cancer results from the sequential accumulation of mutations or deletions in the coding sequence of a number of tumor-suppressor genes and oncogenes, together with aberrant activity of molecules controlling genomic stability (*2*). Patients with familial adenomatous polyposis, a disease characterized by the presence of numerous colorectal polyps, harbor germline mutations of one allele of the *adenomatous polyposis coli* (*APC*) tumor-suppressor gene and develop colon cancer upon mutational damage or loss of the wild-type allele (*3*). Like humans, mice with germline mutations in *APC*, Apc^{min} (multiple intestinal neoplasia) mice, are predisposed to the formation of intestinal adenomas (*4*). Loss of full-length APC proteins is also one of the earliest events occurring in sporadic colon cancer, suggesting that APC may act as a gatekeeper of the colonic epithelium. Nonsteroidal anti-inflammatory drugs (NSAIDs)—which inhibit two enzymes involved in prostaglandin biosynthesis, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)—reduce the number and size of adenomas in patients with familial adenomatous polyposis and prevent colon cancer development in Apc^{min} mice (*5*). Indeed, emerging clinical and experimental evidence now supports a potent antitumorigenic efficacy of NSAIDs in colon cancer (*6*) and implicates the contribution of COX-2 and one of its metabolites, prostaglandin E2 (PGE2), in colon cancer development (*7*). How the

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interplay between PGE2 and APC-regulated pathways leads to colon cancer cell growth remains poorly understood.

PGE2 is a potent mitogen in colon cancer cells (*8*), as reflected by its ability to stimulate the synthesis of DNA to an extent similar to that provoked by serum in DLD-1 cells, a colon cancer cell line homozygous for an inactivating mutation in *APC* (*9*) (fig. S1, A and B) (*10*). We and others (*11*) obtained similar results in a panel of colon cancer–derived cells. Although PGE2 can cause the indirect activation of EGF receptors (EGFR) (*12*), we observed only a minimal increase in the tyrosine phosphorylation of EGFRs upon PGE2 treatment. Furthermore, EGFR kinase inhibitors, such as AG1478, diminished the mitogenic response to EGF but did not prevent the stimulation of DNA synthesis in response to PGE2 or serum (supporting online text). These results indicate that PGE2 may also stimulate EGFR-independent cell growth pathway(s). Among them, we focused our attention on β-catenin, whose cytoplasmic stabilization contributes to colon cancer progression upon *APC* loss (*13*). Increased amounts of β-catenin protein lead to complex formation between β-catenin and members of the transcription factor family that includes the T cell factor (TCF) and lymphoid enhancer factor-1 (LEF) family of DNA-binding proteins, resulting in activation of target gene promoters (*13*).

PGE2 stimulated expression of a β-catenin/TCF/LEF-dependent reporter gene system, TOPflash (*14*, *15*), in colon cancer cells (Fig. 1B and fig. S1C). Similar results were recently reported by others while this study was under revision (*16*). Because PGE2 receptors are coupled to the G protein G_s, which causes accumulation of cyclic adenosine monophosphate (cAMP) and activates protein kinase A (PKA), we confirmed that PGE2 treatment or transfection of cells with the active catalytic subunit of PKA also stimulated the activity of a cAMP-responsive-element–driven reporter gene (CRE-luc) (Fig. 1A). However, PKA did not activate TOPflash (Fig. 1B). Similarly, accumulation of cAMP after activation of adenylyl cyclase with forskolin (*17*) promoted CRE-luc activation but not TOPflash reporter activity (Fig. 1, A and B). These results suggest that activation of PKA is not sufficient to stimulate the β-catenin pathway. Rather, activation of TOPflash by PGE2 correlated with the dephosphorylation of β-catenin and its accumulation and nuclear translocation (Fig. 1C). Furthermore, β-catenin was strictly required for the mitogenic activity of PGE2, because reduction of cellular concentration of β-catenin by a specific small interfering RNA (siRNA) inhibited the growth-promoting effect of this metabolic product of COX-2 (Fig. 1D).

Because EP2 receptors are central mediators of the responses to PGE2 in colon cancer cells (*7*, *18*), we tested whether these prostaglandin receptors promoted activation of β-catenin upon ectopic expression in human kidney embryonic epithelial HEK293T cells. We used β-adrenergic receptors, a prototypical G_s-coupled receptor (*19*), as a control. PGE2 and isoproterenol, a β-adrenergic agonist, stimulated both the CRE and TOPflash reporter systems (Fig. 2A). In contrast, forskolin, which increases intracellular cAMP, did not enhance TOPflash activity despite activating CRE-luc to a similar extent as did PGE2. Because EP2 and β-adrenergic receptors are coupled to G_s proteins, we tested whether the reporter activity could be increased by a constitutive active form of $G\alpha_s$ ($G\alpha_sQ227L$, $G\alpha_sQL$) (Fig. 2B). $G\alpha_sQL$ activated both CRE- and TOPflash-mediated luciferase activity in a dose-dependent manner. Activation of the β-catenin pathway required the constitutive activity of $G\alpha_s$, because expression of its wild-type form, $G\alpha_sWT$, or an active mutant of $G\alpha_{i2}$, $G\alpha_{i2}QL$, failed to stimulate TOPflash activity in HEK293T and colon cancer cells (fig. S2). However, specific inhibition of PKA by expression of PKI, a heat-stable inhibitor of PKA (*20*), abolished the activation of CRE by $G\alpha_sQL$

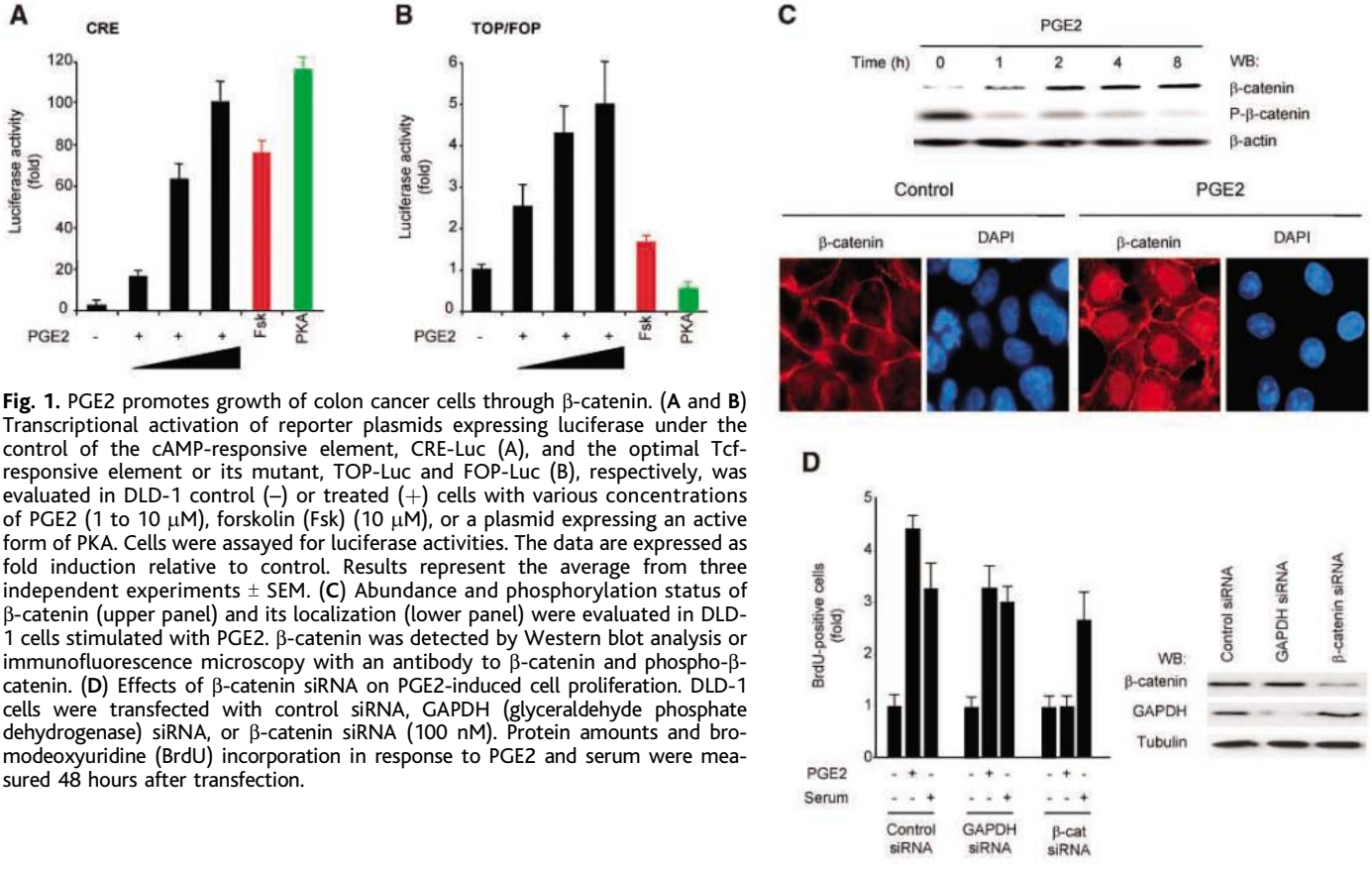

Fig. 1. PGE2 promotes growth of colon cancer cells through β-catenin. (**A** and **B**) Transcriptional activation of reporter plasmids expressing luciferase under the control of the cAMP-responsive element, CRE-Luc (A), and the optimal Tcf-responsive element or its mutant, TOP-Luc and FOP-Luc (B), respectively, was evaluated in DLD-1 control (–) or treated (+) cells with various concentrations of PGE2 (1 to 10 μM), forskolin (Fsk) (10 μM), or a plasmid expressing an active form of PKA. Cells were assayed for luciferase activities. The data are expressed as fold induction relative to control. Results represent the average from three independent experiments ± SEM. (**C**) Abundance and phosphorylation status of β-catenin (upper panel) and its localization (lower panel) were evaluated in DLD-1 cells stimulated with PGE2. β-catenin was detected by Western blot analysis or immunofluorescence microscopy with an antibody to β-catenin and phospho-β-catenin. (**D**) Effects of β-catenin siRNA on PGE2-induced cell proliferation. DLD-1 cells were transfected with control siRNA, GAPDH (glyceraldehyde phosphate dehydrogenase) siRNA, or β-catenin siRNA (100 nM). Protein amounts and bromodeoxyuridine (BrdU) incorporation in response to PGE2 and serum were measured 48 hours after transfection.

and PGE2 but not their activation of TOPflash (Fig. 2B). This result suggested that G_s-coupled receptors activate β-catenin through the $G\alpha_s$ protein but independently of cAMP and PKA.

The pathway leading to β-catenin activation by Wnt involves a complex series of events that results in the dissociation of β-catenin from axin, a scaffold protein that forms a large molecular complex with APC, the signal transducer disheveled (Dsh), and GSK-3β, a kinase that phosphorylates β-catenin, thereby promoting its ubiquitin-dependent proteolytic degradation (*21*). The importance of this inhibitory activity of axin is reinforced by the observation that inactivating mutations in axin are found in hepatocellular carcinomas (*22*). Axin binds APC through a regulator of G protein signaling (RGS) domain (*23*), which is similar in primary amino acid sequence and overall three-dimensional structure to other RGS proteins, whose best known function is to accelerate the guanosine triphosphatase (GTPase) activity of G proteins (*24*). However, the surface area of the axin RGS domain that binds APC is distinct from that used by other RGS proteins to bind G protein α subunits (*25*). This observation and the ability of G_s-coupled receptors to stimulate the transcriptional activity of β-catenin prompted us to explore whether the axin RGS domain may provide a direct link between $G\alpha_s$ and the β-catenin signaling axis. By overexpressing an epitope-tagged form of axin in HEK293T cells (fig. S3A), we observed that axin coimmunoprecipitated with $G\alpha_s$QL but not with the active form of $G\alpha_{i2}$ (Fig. 3, A and B). $G\alpha_s$ wild-type also coimmunoprecipitated with axin, but only when cells were treated with aluminum fluoride, which promotes Gα subunits to acquire a conformation that resembles their transition state, thus favoring RGS binding and GTPase activating protein (GAP) activity (*26*) (Fig. 3B). We next expressed epitope-tagged forms of individual axin domains, including the RGS domain, a region including the GSK-3β phosphorylation and binding sites, a β-catenin binding region, a protein phosphatase 2A (PP2A) binding area, and a DIX domain by which axin binds dsh (Fig. 3C and fig. S3B). Upon coexpression in HEK293T cells, only the RGS domain of axin was coimmunoprecipitated with $G\alpha_s$QL (Fig. 3C), indicating that axin interacts with $G\alpha_s$ through its RGS domain. Indeed, expression of a lentivirus encoding the axin RGS domain fused to green florescent protein (GFP) in DLD1 cells inhibited the activation of TOPflash evoked by PGE2 but not activation by an active mutant of β-catenin (Fig. 3D and fig. S3C). Expression of axin (RGS) also almost completely abolished the mitogenic activity of PGE2 but not the proliferative response to serum in these colon cancer cells (Fig. 3E).

To investigate whether the axin RGS domain binds $G\alpha_s$ directly, we expressed axin (RGS) as a glutathione *S*-transferase (GST)–fusion protein in bacteria and measured in vitro binding to recombinant six-histidine-tagged (H_6)-$G\alpha_s$. GST-RGS bound $G\alpha_s$ immobilized on nickel beads in an aluminum fluoride–dependent manner, as did an RGS protein that binds $G\alpha_s$ specifically, PX1 (RGS) (Fig. 3F, left panels). The guanosine diphosphate (GDP)–loaded, inactive form of $G\alpha_s$ also bound PX1 (RGS), albeit to a much lesser extent, as observed for other RGS proteins in vitro (*27*). By using GTPγS, a non-hydrolyzable GTP analog, we observed that $G\alpha_s$ does not bind to either RGS in its GTP-bound active state. A G_i-specific GAP, RGS19, did not bind $G\alpha_s$ under any condition. Nearly identical results were obtained in reciprocal experiments in which these bacterially expressed GST-RGS fusion proteins were precipitated with glutathione beads (Fig. 3F, left panels). As a control, recombinant $G\alpha_i$ efficiently bound GST-RGS19 in the presence of aluminum fluoride, but not to PX1 or axin (RGS) (Fig. 3F). These findings indicated that $G\alpha_s$ binds directly to the RGS domain of axin in a transition-state conformation.

Because axin bound $G\alpha_s$ in an aluminum fluoride–dependent manner, we evaluated whether axin could increase the GTPase activity of $G\alpha_s$. However, neither the RGS domain of axin nor full-length axin purified from baculovirus-infected Sf9 cells promoted the GTPase activity of $G\alpha_s$ during a single catalytic cycle of the enzyme (fig. S3D). Thus, additional accessory molecules or other modifications of axin could be required for its GAP activity, as is the case for other RGS proteins (*28*). It is also possible that the RGS domain of axin might be used primarily as a structural feature by which this scaffold protein can interact with and act as an effector for $G\alpha_s$, as do the RGS domain–containing RhoGEFs, which are effectors for G proteins of the $G\alpha_{12/13}$ family (*29*).

Because phosphorylation of β-catenin by GSK-3β leads to its rapid ubiquitination and subsequent degradation in the proteosome, inactivation of GSK-3β is often a prerequisite for stimulating the accumulation, nuclear translocation, and functional activity of β-catenin (*30*). Treatment of cells with PGE2 led to the rapid phosphorylation of GSK-3β on serine 9 (Fig. 4A and fig. S4), which inhibits its kinase activity (*31*). PGE2 also stimulated Akt activity in a PI3K-dependent manner (Fig. 4B). Because both PKA and Akt can phosphorylate GSK-3β at this inhibitory site (*32*), we tested whether PKA could mediate GSK-3β phosphorylation in response to PGE2. PKI did not prevent the phosphorylation of GSK-3β provoked by PGE2, but it diminished the GSK-3β phosphorylation induced by forskolin, indicating that PGE2 induces GSK-3β phos-

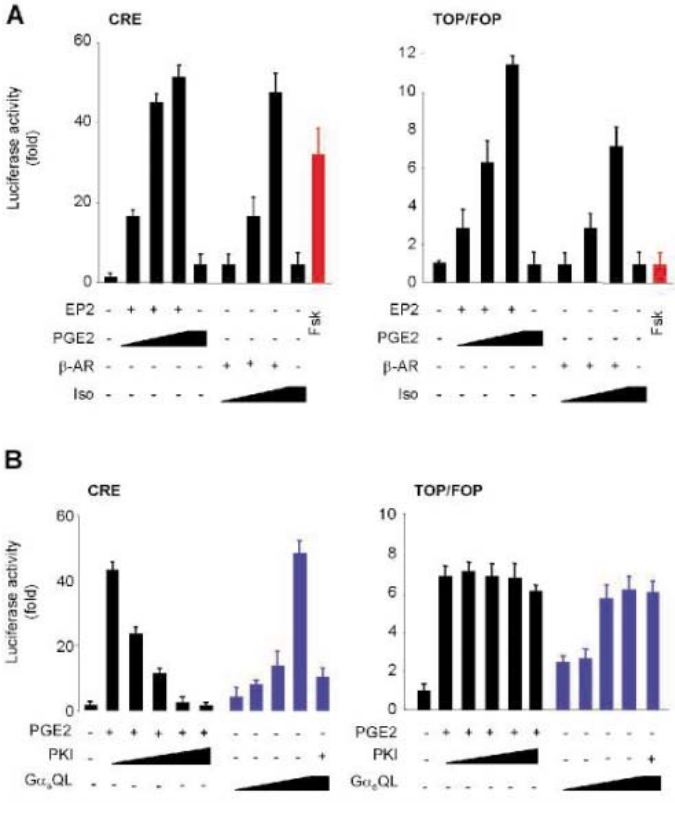

Fig. 2. G_s-coupled receptors (EP2 and β-adrenergic receptors) promote β-catenin activation independently of PKA. (**A**) Empty vector (–) or expression plasmids for EP2 or β-adrenergic receptor were transfected together with pGL3-CRE-Luc or pTOP and pFOP reporter plasmids. After 24 hours, cells were deprived of serum and stimulated with various concentrations of PGE2 (1 to 10 μM), isoproterenol (Iso) (10 to 100 μM), or forskolin (Fsk) (10 μM), as indicated, and assayed for dual luciferase activities. The data are expressed as fold increase relative to control ± SEM of a representative experiment that was repeated three times with nearly identical results. (**B**) TOP and FOP or CRE-Luc activities were also measured in EP2-expressing cells upon transfection of the vector alone or increasing concentrations (0.1 to 1 μg) of pCEFL-$G\alpha_s$QL or RSV-PKI in the absence (–) or presence (+) of PGE2 stimulation (1 μM).

phorylation independently of PKA (Fig. 4A). In contrast, blockade of the PI3K-Akt pathway by wortmannin (WM) abolished both basal and PGE2-induced phosphorylation of GSK-3β (Fig. 4B). Although PGE2 promotes nucleotide exchange on $G\alpha_s$ and subsequent dissociation of GTP-bound $G\alpha_s$ from Gβγ subunits, GTP-bound $G\alpha_s$ does not activate PI3K and Akt (*33*). Thus, we tested whether expression of the C-terminal domain of βark (βARK-C), which causes sequestration of Gβγ subunits (*34*), could inhibit phosphorylation of GSK-3β and Akt. Overexpression of βARK-C did not affect basal phosphorylation of Akt or GSK-3β but abolished the accumulation of their phosphorylated species upon PGE2 stimulation (Fig. 4B). βARK-C did not affect the phosphorylation of Akt and GSK-3β evoked by insulin, and neither WM nor βARK-C affected the activation of the CRE reporter by PGE2 (Fig. 4C), which is dependent on $G\alpha_s$ and cAMP.

Inhibition of GSK-3β phosphorylation by WM or βARK-C only partially reduced the activation of the β-catenin pathway by PGE2 (Fig. 4C), even when both treatments were combined. The view that GSK-3β phosphorylation is required for β-catenin activation has been challenged by experiments using knock-in mice homozygous for both a mutant GSK-3β lacking serine 9 and a mutant for the related GSK-3α lacking serine 21. These mice displayed normal regulation of the Wnt-β-catenin pathway (*35*). Likewise, GSK-3β phosphorylation may not be alone sufficient to stimulate β-catenin, as suggested by the findings that insulin promotes the phosphorylation of GSK-3β but not β-catenin activation (*36*) and that forskolin causes the PKA-dependent phosphorylation of GSK-3β but does not stimulate the TOPflash reporter effectively. Our data suggest that β-catenin stabilization by PGE2 occurs at least through two coordinated mechanisms, one initiated by Gβγ through PI3K, Akt, and the consequent phosphorylation and inactivation of GSK-3β, and another pathway initiated by $G\alpha_s$ that is independent of both GSK-3β phosphorylation and PKA activation.

GSK-3β appears to phosphorylate β-catenin primarily when it is bound to axin (*37*). We observed that GSK-3β coimmunoprecipitated with endogenous axin in both HEK293T and colon cancer cells (Fig. 4, D and E). However, stimulation of cells with PGE2 or expression of activated forms of $G\alpha_s$ was associated with reduced amounts of GSK-3β bound to

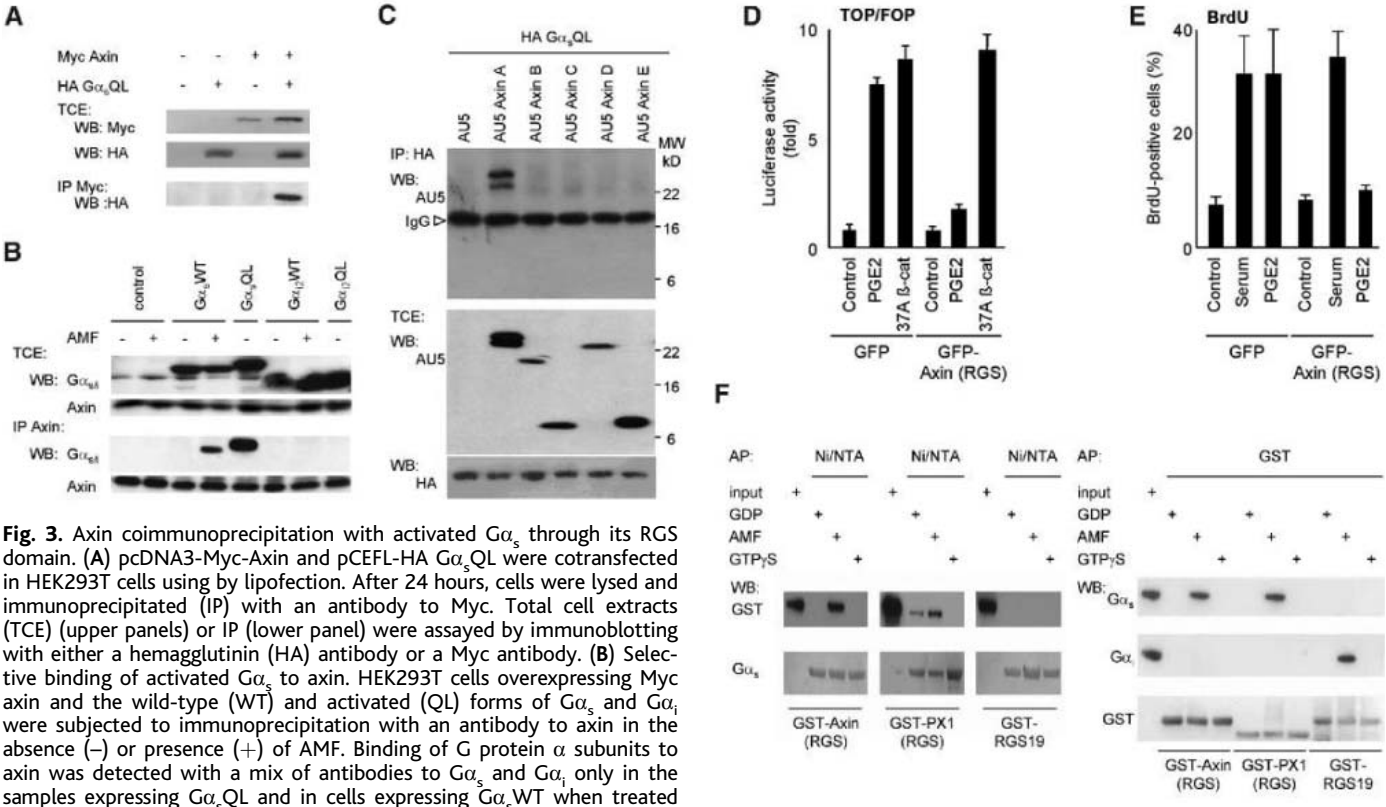

Fig. 3. Axin coimmunoprecipitation with activated $G\alpha_s$ through its RGS domain. (**A**) pcDNA3-Myc-Axin and pCEFL-HA $G\alpha_s$QL were cotransfected in HEK293T cells using by lipofection. After 24 hours, cells were lysed and immunoprecipitated (IP) with an antibody to Myc. Total cell extracts (TCE) (upper panels) or IP (lower panel) were assayed by immunoblotting with either a hemagglutinin (HA) antibody or a Myc antibody. (**B**) Selective binding of activated $G\alpha_s$ to axin. HEK293T cells overexpressing Myc axin and the wild-type (WT) and activated (QL) forms of $G\alpha_s$ and $G\alpha_i$ were subjected to immunoprecipitation with an antibody to axin in the absence (–) or presence (+) of AMF. Binding of G protein α subunits to axin was detected with a mix of antibodies to $G\alpha_s$ and $G\alpha_i$ only in the samples expressing $G\alpha_s$QL and in cells expressing $G\alpha_s$WT when treated with AMF. HA $G\alpha_s$QL was used for these experiments, hence its slightly higher molecular weight. (**C**) Immunoprecipitation of axin RGS domain with $G\alpha_s$QL. Vector-alone (AU5), or epitope-tagged forms of individual axin domains, including the RGS domain (A), a region including the GSK-3β phosphorylation and binding sites (B), a β-catenin binding region (C), a PP2A binding area (D), and a DIX domain by which axin binds dsh (E), were cotransfected with pCEFL-HA $G\alpha_s$QL. After 24 hours, cells were lysed and immunoprecipitated with an antibody to HA and analyzed by Western blotting with antibody to AU5 (upper panel). Total cell extracts were immunoblotted with either AU5 or HA antibody (lower panels). A band corresponding to the anti-HA immunoglobulin G (IgG) is depicted by an empty arrowhead. The position of the molecular size markers is indicated. (**D** and **E**) Axin-RGS domain inhibits the activation of the β-catenin pathway by PGE2, as well as its mitogenic effect. (D) Luciferase activity was measured in GFP and GFP-Axin RGS–infected DLD-1 cells transfected with the TOP and FOP reporter plasmids and stimulated with PGE2 (1 μM). Luciferase expression in cell lysates is represented as in Fig. 1. Transfection of an activated mutant of β-catenin (37A β-cat) was used as a control. (E) DNA synthesis was also measured as described in Fig. 1. The data were averaged from three independent experiments ± SEM, in which at least 500 cells were counted. (**F**) In vitro binding of axin to activated $G\alpha_s$ but not $G\alpha_i$. Left: Recombinant $His_6G\alpha_s$ was immobilized on Ni^{++} agarose (Ni/NTA) in the presence of GDP, aluminum magnesium fluoride (AMF), or GTPγS, and incubated with recombinant GST-Axin (RGS domain), GST-PX1 (RGS), or GST-RGS19 (GAIP). After bead washing, bound proteins were identified by immunoblotting with antibody to $G\alpha_s$ (top) or antibody to GST (bottom), running half of the total purified GST-fusion protein used for the experiment (input) as a control. Right: The indicated bacterially expressed GST-fusion proteins were purified and incubated in vitro with $His_6G\alpha_s$ or $G\alpha_{i1}$ and affinity-precipitated with glutathione agarose. Recombinant proteins bound to beads after extensive washing were detected with the indicated antibodies. Half of the total purified $His_6G\alpha_s$ or $G\alpha_{i1}$ used for the experiment (input) was run as a control. Figures represent four similar experiments.

axin without affecting the total amount of GSK-3β (Fig. 4, D and E). Because the GSK-binding site on axin is close to the RGS domain, we tested whether $G\alpha_s$ interaction with axin competes for GSK-3β binding in experiments with recombinant proteins. However, a 10-fold molar excess $G\alpha_s$-AMF (aluminum magnesium fluoride) did not reduce association of axin-H_6 with recombinant GSK-3β, suggesting that additional factors may be required for the dissociation of axin-GSK-3β complexes.

To test whether displacing GSK-3β from axin is sufficient to stimulate the β-catenin pathway, we first reduced the cellular concentration of GSK-3β and axin by specific siRNAs. Knockdown of axin or GSK-3β activated the TOPflash reporter (Fig. 5A and fig. S5A). Furthermore, the GSK-3 binding region of axin, but not the axin RGS as a control, bound GSK-3β and displaced it from its binding to axin (Fig. 5B and fig. S5B). This resulted in an increase in β-catenin activity similar to that stimulated by PGE2. Thus, the ability of PGE2 and $G\alpha_s$ to promote the release of GSK-3β from axin-containing complexes may represent an alternative mechanism to inhibit the functional activity of GSK-3β independently of phosphorylation.

These observations may have important implications for the study of β-catenin activation by Wnt, which involves two cell surface receptors, an LDL-containing single-pass transmembrane protein, LRP5 or LRP6, and a seven-transmembrane receptor, Frizzled (*38*, *39*). Whereas LRP5 and LRP6 appear to bind axin directly, how Frizzled signals to the canonical β-catenin pathway is still unclear. Frizzled may activate heterotrimeric G proteins (*40*), thus raising the possibility that the association of G protein α subunits with the RGS domain of axin may represent a point of convergence between the Wnt and prostaglandin-initiated pathways leading to β-catenin activation. On the other hand, how this process can be regulated by APC is at the present unknown. In our in vitro experiments, a peptide containing the primary sequence of APC that binds to axin RGS (*23*) did not compete for $G\alpha_s$ binding to axin (RGS). This is consistent with a distinct binding surface area for APC and $G\alpha_s$ on the axin RGS domain as predicted by the crystal structure (*25*). In contrast, expression of APC in colon cancer cells inhibited the activation of the β-catenin reporter system by PGE2. Considering that the tumor-promoting effect of PGE2 becomes evident only in the absence of functional APC, it is conceivable that APC may act by limiting the activation of β-catenin by prostaglandins, which are normally released in the colon in response to bacterial infection and proinflammatory substances. APC may bind the axin RGS domain and hinder the access of Gα subunits to this RGS. Alternatively, as APC interacts with β-catenin through numerous binding motifs, it may sequester free β-catenin, once it has been released from axin, and promote its turnover (*41*), thus raising the threshold of EP2 activation that is required to stimulate β-catenin–dependent gene expression.

Our studies indicate that in the absence of a functional APC, PGE2 can stimulate the proliferation of colon cancer cells by activating the β-catenin axis through a biochemical pathway initiated by the activation of the G protein–linked PGE2 receptor, EP2 (Fig. 6). PGE2 stimulation leads to the association of the activated α subunit of G_s with the RGS domain of axin, promoting release of GSK-3β from its complex with axin. Concurrently, free Gβγ subunits liberated upon $G\alpha_s$ activation

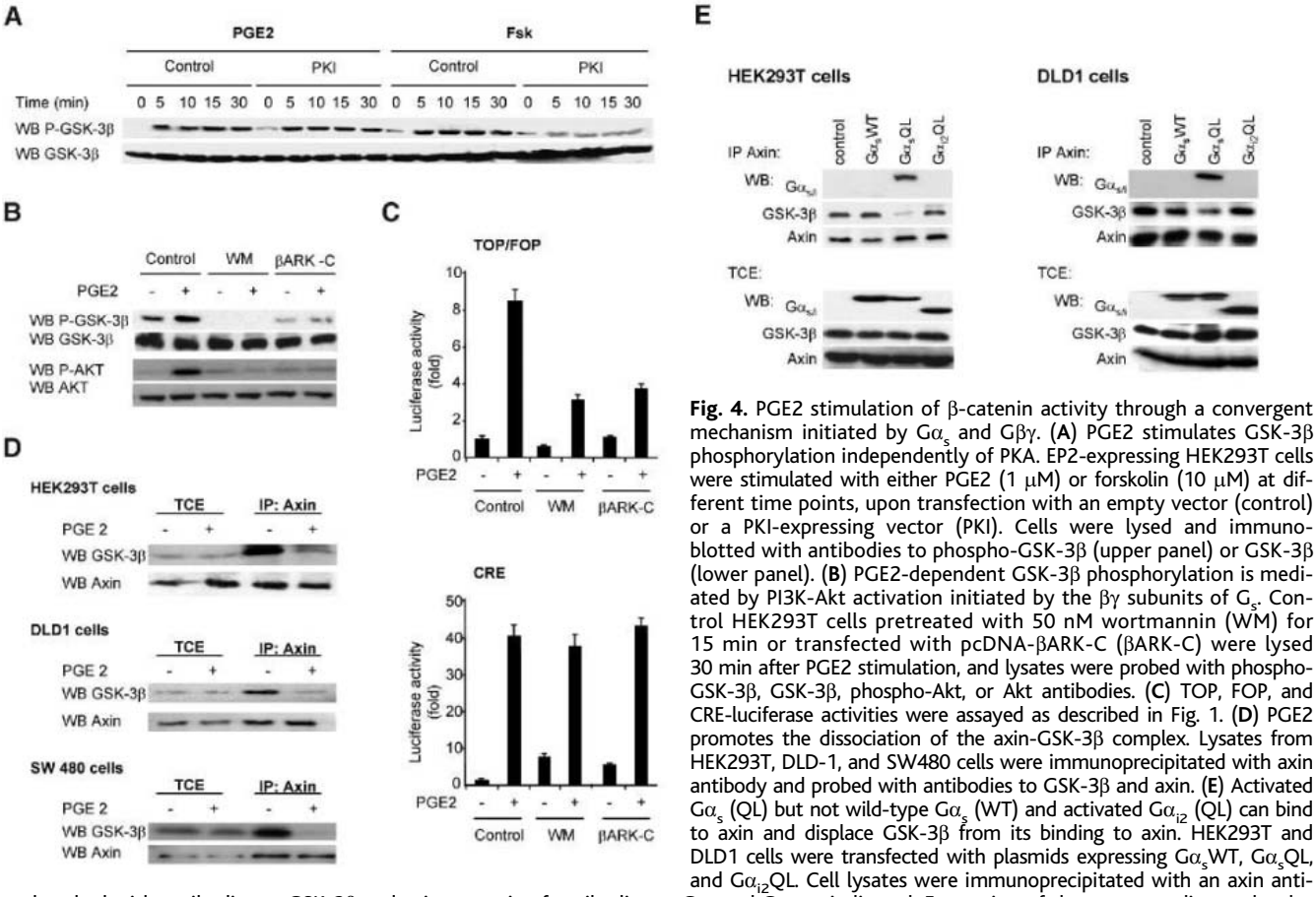

Fig. 4. PGE2 stimulation of β-catenin activity through a convergent mechanism initiated by $G\alpha_s$ and Gβγ. (**A**) PGE2 stimulates GSK-3β phosphorylation independently of PKA. EP2-expressing HEK293T cells were stimulated with either PGE2 (1 μM) or forskolin (10 μM) at different time points, upon transfection with an empty vector (control) or a PKI-expressing vector (PKI). Cells were lysed and immunoblotted with antibodies to phospho-GSK-3β (upper panel) or GSK-3β (lower panel). (**B**) PGE2-dependent GSK-3β phosphorylation is mediated by PI3K-Akt activation initiated by the βγ subunits of G_s. Control HEK293T cells pretreated with 50 nM wortmannin (WM) for 15 min or transfected with pcDNA-βARK-C (βARK-C) were lysed 30 min after PGE2 stimulation, and lysates were probed with phospho-GSK-3β, GSK-3β, phospho-Akt, or Akt antibodies. (**C**) TOP, FOP, and CRE-luciferase activities were assayed as described in Fig. 1. (**D**) PGE2 promotes the dissociation of the axin-GSK-3β complex. Lysates from HEK293T, DLD-1, and SW480 cells were immunoprecipitated with axin antibody and probed with antibodies to GSK-3β and axin. (**E**) Activated $G\alpha_s$ (QL) but not wild-type $G\alpha_s$ (WT) and activated $G\alpha_{i2}$ (QL) can bind to axin and displace GSK-3β from its binding to axin. HEK293T and DLD1 cells were transfected with plasmids expressing $G\alpha_s$WT, $G\alpha_s$QL, and $G\alpha_{i2}$QL. Cell lysates were immunoprecipitated with an axin antibody and probed with antibodies to GSK-3β and axin or a mix of antibodies to $G\alpha_s$ and $G\alpha_i$, as indicated. Expression of the corresponding molecules was also evaluated in total cell extracts (TCE).

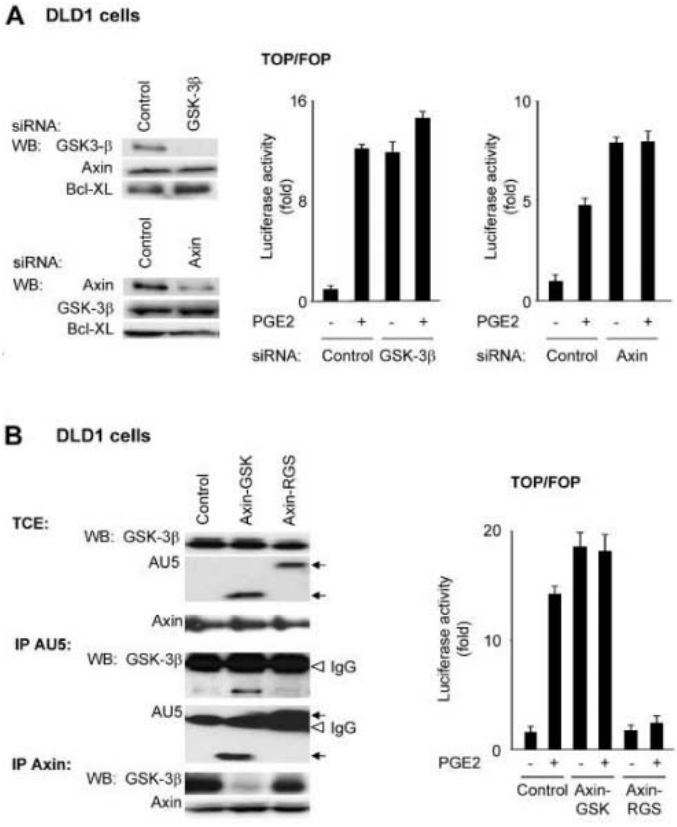

Fig. 5. Knockdown of axin or GSK-3β, or displacement of GSK-3β from axin, is sufficient to stimulate the β-catenin pathway in DLD1 colon cancer cells. (**A**) Knockdown of GSK-3β or axin in DLD1 cells stimulates β-catenin to a similar extent as PGE2 stimulation. Cells were transfected with the indicated siRNAs, and the expression of endogenous axin or GSK-3β was evaluated by Western blot analysis (left panels). Western blot analysis of Bcl-xL was used as a control. Luciferase expression was measured in cells transfected with the TOP and FOP reporter plasmids and the indicated siRNAs, with (+) or without (–) stimulation with PGE2 (1 μM), as in Fig. 1C (right panels). (**B**) Expression of the isolated GSK-3β binding region of axin is sufficient to displace GSK-3β from axin and stimulate β-catenin signaling. The GSK-3β binding region of axin [depicted as (B) in fig. S3] binds GSK-3β in vivo and displaces it from its binding to endogenous axin (left panel). The axin RGS domain [depicted as (A) in fig. S3)], which does not bind GSK-3β, served as a control. Arrows point to the epitope-tagged forms of the indicated axin regions. A band corresponding to the anti-HA IgG is depicted by an empty arrowhead. Cells were transfected with the TOP and FOP reporter plasmids together with the vector control or the expression vectors for the isolated axin domains and either left untreated (–) or stimulated with PGE2 (1 μM) (+). Luciferase expression was measured and represented as in Fig. 1B (right panel).

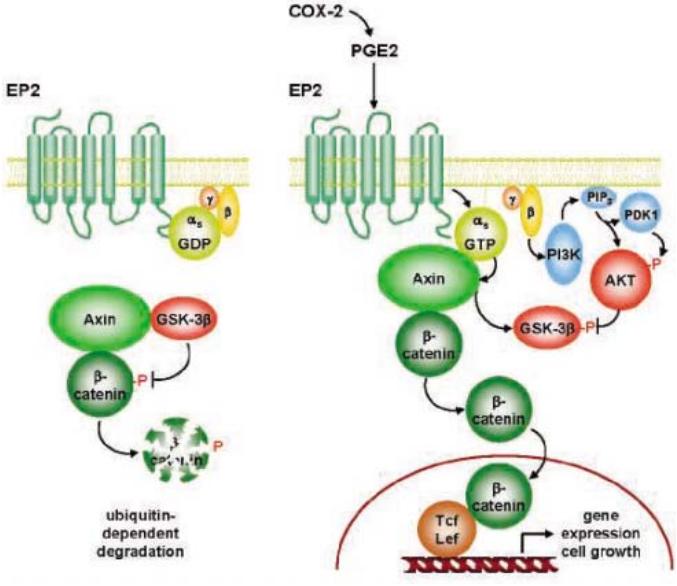

Fig. 6. Schematic representation of β-catenin pathway activation in response to PGE2. In the basal state (left panel), a cytoplasmic protein complex containing GSK-3β and axin promotes the inhibitory phosphorylation and consequent ubiquitin-dependent degradation of β-catenin in the proteasome. Overexpression of COX-2 in colon cancer cells and during inflammatory processes leads to the production of PGE2, which can activate EP2 receptors that are coupled to heterotrimeric G proteins of the G_s family (right panel). Upon exchange of GDP for GTP, the α subunit of G_s binds the RGS domain of axin, thereby promoting the release of GSK-3β from the complex. Concomitantly, free βγ subunits stimulate the PI3K-PDK1-Akt signaling route, which causes the phosphorylation and inactivation of GSK-3β. These events lead to the stabilization and nuclear translocation of β-catenin and to the expression of growth-promoting genes regulated by the Tcf and LEF family of transcription factors.

directly stimulate the activity of PI3K and Akt, leading to phosphorylation and inactivation of GSK-3β. Ultimately, these processes result in the stabilization and nuclear translocation of β-catenin, thereby stimulating LEF and β-catenin–dependent gene expression and the aberrant growth of colon cancer cells. These findings support the existence of a direct molecular mechanism by which COX-2 and inflammation can promote the progression of colon cancer, thus providing a molecular framework for the future clinical evaluation of NSAIDs as chemopreventive strategies for this devastating disease.

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Divergent Immunoglobulin G Subclass Activity Through Selective Fc Receptor Binding

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Subclasses of immunoglobulin G (IgG) display substantial differences in their ability to mediate effector responses, contributing to variable activity of antibodies against microbes and tumors. We demonstrate that the mechanism underlying this long-standing observation of subclass dominance in function is provided by the differential affinities of IgG subclasses for specific activating IgG Fc receptors compared with their affinities for the inhibitory IgG Fc receptor. The significant differences in the ratios of activating-to-inhibitory receptor binding predicted the in vivo activity. We suggest that these highly predictable functions assigned by Fc binding will be an important consideration in the design of therapeutic antibodies and vaccines.

Antibodies have evolved into classes with specific assigned functions. Within these classes, further subclassification extends immunoglobulin diversity, most strikingly in the four subclasses of IgG of mammals (*1*). In rodents and primates, these subclasses have evolved specialized effector responses, such as cytotoxicity, phagocytosis, and release of inflammatory mediators (*2*, *3*). IgG subclass expression is influenced by multiple factors, including the prevailing cytokine environment. For example, the T helper cell T_H2 cytokine interleukin 4 (IL-4) preferentially induces switching to IgG1 and IgE, whereas transforming growth factor–β (TGF-β) induces switching to IgG2b and IgA (*4*–*6*). Alternatively, T_H1 cytokines such as interferon-γ (IFN-γ) result in IgG2a, 2b, and 3 switching (*7*). Switching is also strongly influenced by the nature of the stimulating antigen. For example, protein antigens elicit a thymus-dependent response generally dominated by IgG1, 2a, and 2b, whereas carbohydrate antigens can induce so-called thymus-independent responses that result in IgG3 antibody expression (*8*). Among IgG subclasses, IgG2a and 2b are generally considered to be the most potent for activating effector responses and dominate antiviral immunity and autoimmune diseases (*9*–*11*). Such functional distinctions among these IgG subclasses have been attributed to differences in their capacity to fix complement (*12*, *13*). However, studies in complement-deficient mice have challenged this assumption and have focused attention on the cellular receptors for IgG, the FcγRs, as the primary mediators of IgG effector responses (*14*, *15*). We hypothesized that unified mechanisms accounting for the different potencies of IgG subclasses might be based on the differential binding to the known FcRs.

Activation FcγRs are expressed on all myeloid cells, and their cross-linking results in sustained cellular responses (*3*). Balancing these activation receptors is the inhibitory Fc receptor, FcγRIIB, which, when coligated to activation receptors, dampens the cellular response (*3*, *16*). The coexpression of activation and inhibitory receptors establishes a threshold for cellular triggering by IgG antibodies. Although all FcγRs can bind IgG immune complexes, we have observed that individual Fc receptors display significantly different affinities for IgG subclasses (*17*). We described this differential affinity for functionally distinct FcRs by specific IgG subclasses as a ratio, referred to as the activating-to-inhibitory (or A/I) ratio (*17*). These A/I ratios were found to differ by several orders of magnitude between IgG subclasses and thus raised the possibility that the variation in in vivo IgG subclass activity could be directly linked to the specific A/I ratio. To address this hypothesis, we established an in vivo system for testing antibodies that differed in their A/I ratios. The variable portions of the immunoglobulin heavy chain (V_H regions) of the cloned hybridomas that recognize either the melanosome gp75 antigen (TA99) or a platelet integrin antigen (6A6) were grafted onto IgG1, 2a, 2b, or 3 Fc regions and coexpressed with the appropriate light chains (*17*, *18*). These recombinant antibodies were purified, and subsequent testing revealed that switching the constant regions of IgG did not affect antigen binding affinity (*18*) (table S1). As expected, however, specific differences in binding affinity of each subclass to specific FcγRs were observed, resulting in different A/I ratios for each subclass (*17*) (fig. S1, table S2).

To determine whether the differences in A/I ratios for individual subclasses correlate to in vivo biological activity, we investigated the ability of these class-switched antibodies to mediate their specific biological functions: tumor clearance and platelet depletion (*14*, *19*). Both TA99 (Fig. 1, A and B) and 6A6 (Fig. 1C) carrying IgG2a constant regions (A/I = 70) displayed enhanced activity compared with these antibodies bearing IgG1 constant regions (A/I = 0.1). IgG2a and 2b were equivalent in their ability to mediate platelet clearance, whereas IgG2a resulted in enhanced antibody-dependent cellular cytotoxicity (ADCC) in the metastatic melanoma model compared with IgG2b (A/I = 7) (Fig. 1, A and B). The hierarchy of activity for the IgG subclasses in these immune functions was thus IgG2a ≥ IgG2b > IgG1 ≫ IgG3, mirroring the hierarchy based on the A/I ratios (Fig. 1D).

We next tested the mechanism of this observed differential activity by repeating the experiments using strains of mice carrying specific deficiencies in, or blocked activation of, different activating FcγRs or complement components (Fig. 2; fig. S2). No differences in in vivo activity were observed in complement-deficient (C4, C3, or CR1/2) strains (fig. S2). In contrast, IgG1, 2a, and 2b all depended on expression of activating FcγR, because activity was abrogated in the common γ chain–deficient background (Fig. 2, A, B, and E). Because IgG2a has been shown to bind to all of the γ chain–containing activating FcγRs in vitro [with high affinity (10^8 to 10^9 M^{-1}) to FcγRI, intermediate affinity (10^7 M^{-1}) to FcγRIV, and low affinity (10^6 M^{-1}) to FcγRIII], its in vivo capacity to deplete platelets or to mediate ADCC could, in principle, result from engagement of one or more of these FcγRs. We tested the contributions of each of these

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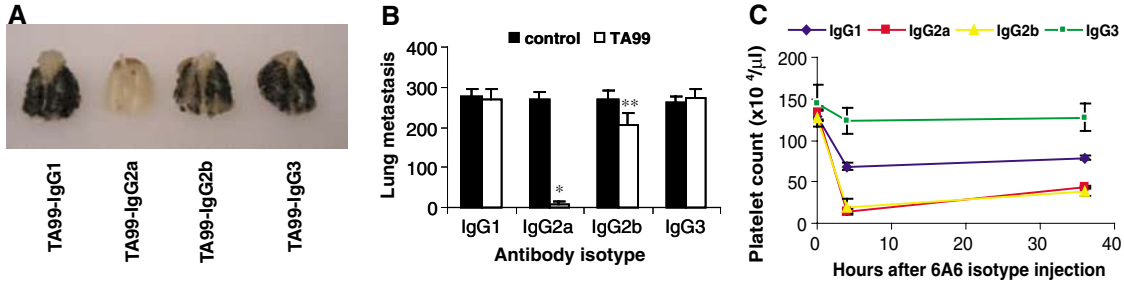

Fig. 1. Hierarchy of antibody isotype–mediated effector functions in vivo. (**A** and **B**) B16-F10 lung metastasis in mice treated with TA99 switch variants (mean ± SEM). Mice were injected as described (*18*); the number of surface lung metastasis was evaluated on day 15. $*P < 0.0001$; $**P < 0.01$. (**C**) Platelet depletion with 6A6 switch variants (mean ± SEM). Mice were injected as described (*18*), platelet counts were determined at the indicated time points. (**D**). A/I ratio for the indicated switch variants.

D

	IgG1 (III/IIB)	IgG2a (IV/IIB)	IgG2b (IV/IIB)
A/I ratio	0.1	69	7

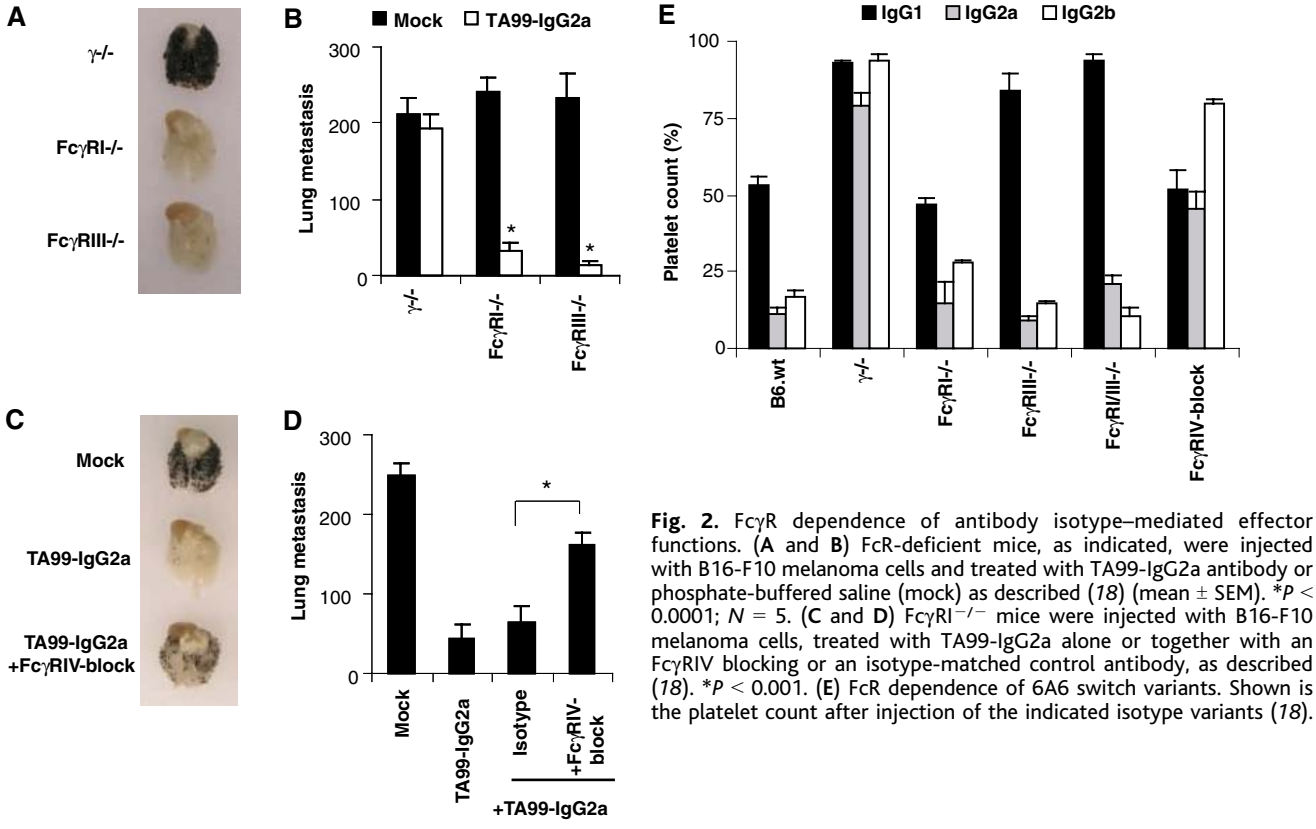

Fig. 2. FcγR dependence of antibody isotype–mediated effector functions. (**A** and **B**) FcR-deficient mice, as indicated, were injected with B16-F10 melanoma cells and treated with TA99-IgG2a antibody or phosphate-buffered saline (mock) as described (*18*) (mean ± SEM). $*P < 0.0001$; $N = 5$. (**C** and **D**) FcγRI$^{-/-}$ mice were injected with B16-F10 melanoma cells, treated with TA99-IgG2a alone or together with an FcγRIV blocking or an isotype-matched control antibody, as described (*18*). $*P < 0.001$. (**E**) FcR dependence of 6A6 switch variants. Shown is the platelet count after injection of the indicated isotype variants (*18*).

receptors to the in vivo activity of this subclass using mice deficient in or blocked for each receptor. TA99-IgG2a–mediated tumor clearance and 6A6-IgG2a–mediated platelet depletion were undiminished in mice deficient in either FcγRI or III (Fig. 2, C to E); in contrast, blocking FcγRIV binding with a specific monoclonal antibody significantly reduced these IgG2a-mediated activities (Fig. 2, C to E). Similarly, 6A6-IgG2b activity was only dependent on FcγRIV engagement, despite its in vitro binding to both FcγRIII and IV (*17*) (Fig. 2E). In contrast, the 6A6-IgG1 switch variant was only dependent on FcγRIII engagement (*17*) (Fig. 2E).

The A/I ratio of 0.1 for IgG1 suggested that IgG1 might exhibit a greater dependence in its in vivo activity on FcγRIIB expression. Consistent with this prediction, we observed that IgG1 displayed the most significant enhancement in activity in mice lacking the inhibitory receptor, both in tumor clearance (Fig. 3, A and B) and platelet depletion (Fig. 3C) relative to IgG2a or 2b switch variants (Fig. 3, A to C). In contrast, IgG2a (A/I = 70) showed the least enhancement in biological activity in FcγRIIB-deficient mice (Fig. 3, B and C). By comparison, IgG2b (A/I = 7) differed in the magnitude of enhancement displayed in the FcγRIIB-deficient strains between each of the two models, with a significant increase in tumor clearance (Fig. 3B), but only minimal enhancement in platelet depletion (Fig. 3C). The intermediate A/I ratio of this subclass may render it more sensitive to the absolute level of inhibitory receptor surface expression and the specific effector cell engaged. This makes the dependence of IgG2b on FcγRIIB consistent with the observation that expression of this FcγR is minimal on splenic macrophages (*15*, *20*), the cell type responsible for platelet clearance, but higher on alveolar macrophages, which are involved in the metastatic melanoma model (*21*).

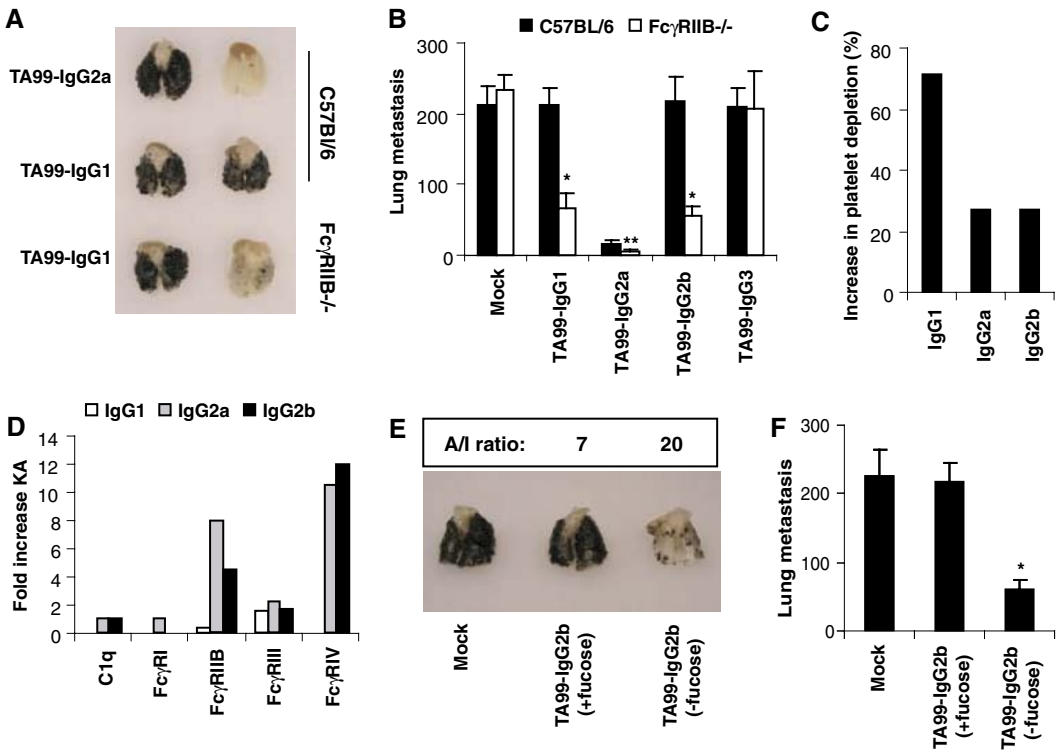

Fig. 3. A/I ratio determines in vivo efficacy of native and modified antibodies. (**A** to **C**) Differential effects of inhibitory receptor expression on IgG subclass activity. (A and B) C57BL/6 wild-type or FcγRIIB$^{-/-}$ mice were injected with B16-F10 melanoma cells and treated with TA99 switch variants, as described (*18*). *P < 0.0001; **P < 0.05; N = 5. (C) C57BL/6 or FcγRIIB$^{-/-}$ mice were injected with 6A6 antibody switch variants, as described (*18*). (**D** to **F**) Modified antibodies with increased A/I ratio display enhanced cytotoxic activity. (D) Fold increase in association constants (K_A) for C1q and FcγRs I-IV in binding to fucosylated or non-fucosylated TA99 switch variants. (E and F) Clearance of B16 melanoma lung metastasis with TA99-IgG2b with or without fucose (*18*). *P < 0.0001.

To further explore the relation between the A/I ratio of IgG subclasses and their activity, modified IgG constant regions were generated. FcR binding to IgG depends on the presence of N-linked glycosylation at position 297, and deglycosylation abrogates all FcR binding (*22*). However, selective removal of specific carbohydrates, such as fucose, has been suggested to enhance human IgG1 binding to human FcγRIII and, thus, to enhance NK cell–mediated ADCC in vitro (*23*, *24*). We therefore prepared fucose-sufficient and fucose-deficient TA99-IgG1, 2a, and 2b subclasses and compared their binding to antigen, complement, and FcγRI, II, III, and IV (*18*). Fucose-deficient antibodies ranged in their binding affinities to their respective FcγRs, but not for antigen or C1q, the first component of complement (*23*) (table S1, S2; Fig. 3D). TA99-IgG1, with or without fucose, displayed minimal differences in binding to FcγRIIB and III, whereas fucose-deficient IgG2a and 2b antibodies bound with higher affinity (by an order of magnitude) to FcγRIIB and IV compared with fucose-sufficient versions. These differences in binding affinities resulted in alterations to the respective A/I ratios that were most pronounced for IgG2b, with defucosylation increasing its A/I ratio from 7 to 20 (Fig. 3E; table S2). Furthermore, this translated into significantly enhanced in vivo activity for fucose-deficient TA99-IgG2b (Fig. 3, E and F). This selective effect of IgG defucosylation on FcR binding further illustrates the specificity of IgG subclasses in their interactions with individual FcRs and the contribution of these affinities for IgG to in vivo activity.

The studies described here provide a mechanistic basis for the observed variation in IgG subclass activity in both active and passive vaccination and in the variable pathogenicity of the IgG subclasses in autoimmune conditions. The selective FcR binding affinities for the IgG subclasses and fucose-deficient antibodies appeared to be predictive of the in vivo activity for cytotoxic antibodies in models of tumor clearance and platelet depletion. Although significant differences between the mouse and human IgG subclasses and the FcγRs have been described (*25*), the principles that have emerged from these mouse studies are likely to apply to human antibodies as well as their respective FcRs. Such considerations may prove important in the design of antibody-based therapeutics and active vaccination protocols.

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Structural Roles for Human Translation Factor eIF3 in Initiation of Protein Synthesis

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Protein synthesis in mammalian cells requires initiation factor eIF3, a ~750-kilodalton complex that controls assembly of 40*S* ribosomal subunits on messenger RNAs (mRNAs) bearing either a 5′-cap or an internal ribosome entry site (IRES). Cryo–electron microscopy reconstructions show that eIF3, a five-lobed particle, interacts with the hepatitis C virus (HCV) IRES RNA and the 5′-cap binding complex eIF4F via the same domain. Detailed modeling of eIF3 and eIF4F onto the 40*S* ribosomal subunit reveals that eIF3 uses eIF4F or the HCV IRES in structurally similar ways to position the mRNA strand near the exit site of 40*S*, promoting initiation complex assembly.

Protein synthesis begins with recruitment of an mRNA to the small ribosomal subunit before the formation of active ribosomes (*1*). In mammalian cells, translation initiation factor eIF3 binds 40*S* subunits and recruits mRNAs bearing a methylated guanosine cap at the 5′-end (5′-m7G cap) via direct interaction with the 5′-cap binding complex eIF4F (*1*, *2*). Many viral mRNAs, however, use a structured RNA element, or IRES, that interacts with eIF3 and functionally replaces the mRNA 5′-cap and all or part of the cap binding complex (*3*). Comprising at least 12 proteins in humans, eIF3 also prevents premature association of the 40*S* and 60*S* ribosomal subunits, interacts with other initiation factors that detect the start codon, and helps assemble active ribosomes (*1*, *2*). Despite its importance in both cellular and viral protein synthesis initiation, the structural bases for eIF3 activities and interactions with the translational machinery are unknown.

To obtain a structure of eIF3, we produced an initial electron microscopy (EM) reconstruction using the random-conical tilt method on negatively stained human eIF3 samples (*4*, *5*). This reconstruction was then used as a reference for projection matching of cryo-EM data to produce an improved reconstruction at ~30 Å resolution (Fig. 1A) (*5*, *6*). The largest dimension of eIF3 is ~175 Å, with individual domains ranging between 60 and 100 Å in length. eIF3 shows anthropomorphic features that have been used to name the five domains according to body parts, including a head, arms, and legs (Fig. 1A). The front surface of the complex is rather flat, whereas the back contains a cleft separating the domains into two slabs of density.

This structure must interact with RNA and protein factors to organize the assembly of the translation initiation complex. In the hepatitis C virus, the uncapped mRNA contains an IRES in its 5′-untranslated region that binds directly to eIF3 to form part of the ternary complex necessary for viral protein synthesis (*7*–*9*). The IRES circumvents the need for eIF3 binding to initiation factor eIF4G, a component of eIF4F, which is essential to position the 5′-end of the transcript on the ribosome during translation initiation on most cellular mRNAs (*10*).

We used cryo-EM and difference mapping to locate the HCV IRES binding site on eIF3. Initial two-dimensional (2D) analysis of eIF3-IRES showed extra density extending from the left arm of eIF3 (Fig. 1B, left panel) (*5*). We used projection-matching to the unliganded eIF3 structure, together with common-line cross-correlation between class averages corresponding to different views, to assign the particles to one of three IRES conformational groups (*5*, *11*). From this analysis, three reconstructions were produced representing the full range of observed IRES conformations (Fig. 1B). One of these reconstructions, corresponding to the most abundant IRES conformer (Fig. 1B, conformer-1), shows a hook-like density for the IRES protruding from the left arm of eIF3. The difference map between refined conformer-1 and unliganded eIF3 corresponds well to the HCV IRES density observed in a previous reconstruction of the HCV IRES-40*S* complex (Fig. 2A) (*5*, *12*). This analysis reveals that the IRES extends across eIF3, from the left arm to the right leg (Fig. 2A). On the basis of previous mapping of RNA helices onto the IRES density, domain IIId/e/f of the IRES appears to be located near the central region of eIF3, domain IIIa/b/c is near the right leg, and domain II corresponds to the flexible region emanating from the left arm of eIF3 (*12*). The extended interaction surface between the

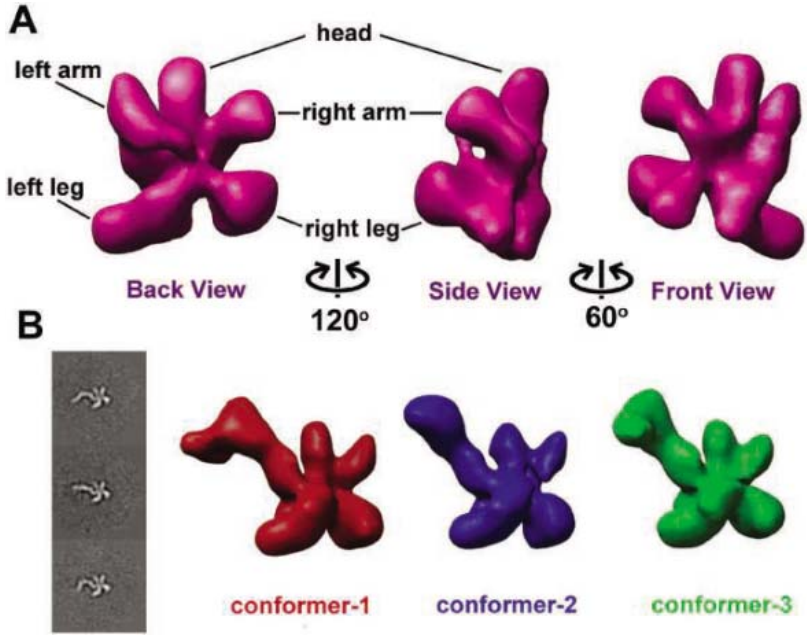

Fig. 1. Cryo-EM reconstructions of eIF3 and eIF3-IRES. (**A**) eIF3 at ~30 Å resolution [by 0.5 Fourier shell correlation function (FSC) cutoff]. (**B**) eIF3-IRES class averages showing flexible HCV-IRES (left) and corresponding 3D models (back view of eIF3 toward the viewer). The refined conformer-1 was used for difference mapping and modeling.

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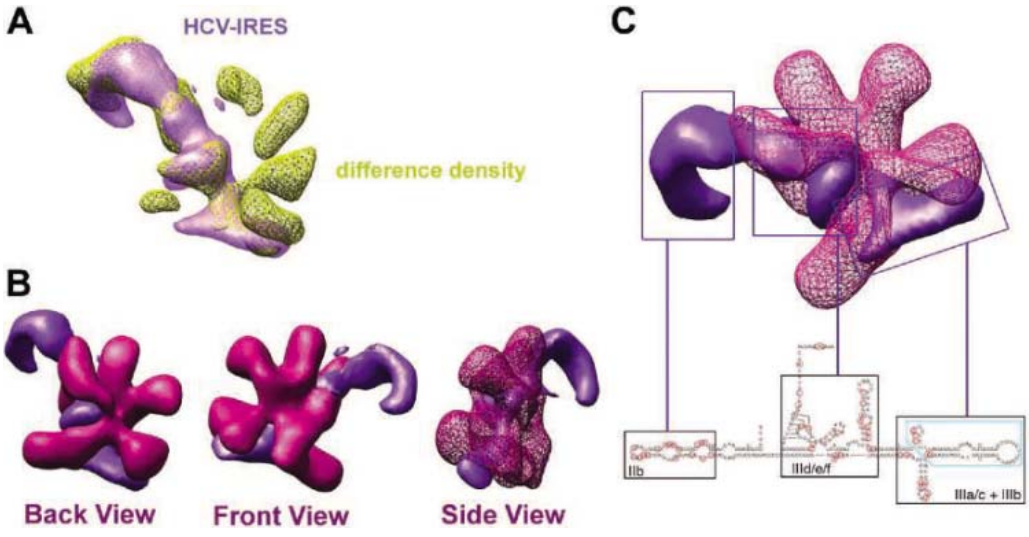

Fig. 2. Mapping of IRES domain interactions with eIF3. (**A**) Difference map between eIF3-IRES conformer-1 (Fig. 1B) and eIF3 (yellow mesh) and its superposition with the HCV-IRES density from the 40*S*-IRES study (transparent blue surface) (*12*). (**B**) Composite of eIF3 density and HCV-IRES density. (**C**) Predicted position of IRES domains in relation to eIF3 structure, based on previous mapping (*12*).

IRES and eIF3 agrees with biochemical studies in which at least four subunits of eIF3 (eIF3a, -b/c, -d, and -f) could be cross-linked to the IRES (*7*). Modification interference and footprinting assays that identified the IRES domain IIIa/b/c (Fig. 2C) as the primary binding site for eIF3 may have underrepresented weaker or more dynamic interaction sites (*7*, *13*). Notably, the difference map also includes extra densities (Fig. 2A) that suggest conformational changes in the right arm and right leg domains of eIF3 upon IRES binding.

By superimposing the structure of eIF3-IRES (Fig. 2B) onto a previous cryo-EM reconstruction of 40*S*-IRES using the IRES density, we produced a model of the 40*S*-eIF3-IRES complex that illustrates the interaction of eIF3 with 40*S* (Fig. 3, A and B) (*12*). This ternary model lacks any physical overlap of the components, showing excellent surface complementarity between eIF3 and the functionally critical 40*S* platform. The location and orientation of eIF3 on 40*S* is further supported by 2D difference mapping of negatively stained 40*S*-eIF3 complex and 40*S* alone (Fig. 3C) and by a previous low-resolution reconstruction of rabbit 40*S*-eIF3 (*5*, *14*). The eIF3 mass in the ternary complex localizes to the solvent-exposed side of 40*S*, where the front surface of eIF3 contacts 40*S*. The front of the eIF3 left leg sits below the platform near the 60*S* subunit interface, whereas the left arm points toward the tRNA exit (E) site (Fig. 3, A and B). The relative positions of 40*S*, eIF3, and IRES in our model (Fig. 2B, side view; Fig. 3, A and B) make it unlikely that IRES binding to 40*S* or eIF3 takes place after 40*S*-eIF3 binding.

The position of eIF3 in the 40*S*-eIF3-IRES model provides a plausible explanation for its role in preventing premature joining of 40*S* and 60*S* subunits. Structural and biochemical studies of the bacterial and eukaryotic ribosomes revealed two critical intersubunit contacts (B4 and B2a) involving binding of the small subunit protein S15/rpS13 to helix 34 of the large subunit (B4) and interaction between the small subunit helix 44 and helix 69 of the large subunit (B2a) (*15*–*17*). Our model suggests that the left toe of eIF3 covers the small subunit protein S15/rpS13, likely preventing the helix 34 contact (Fig. 3B). Fur-

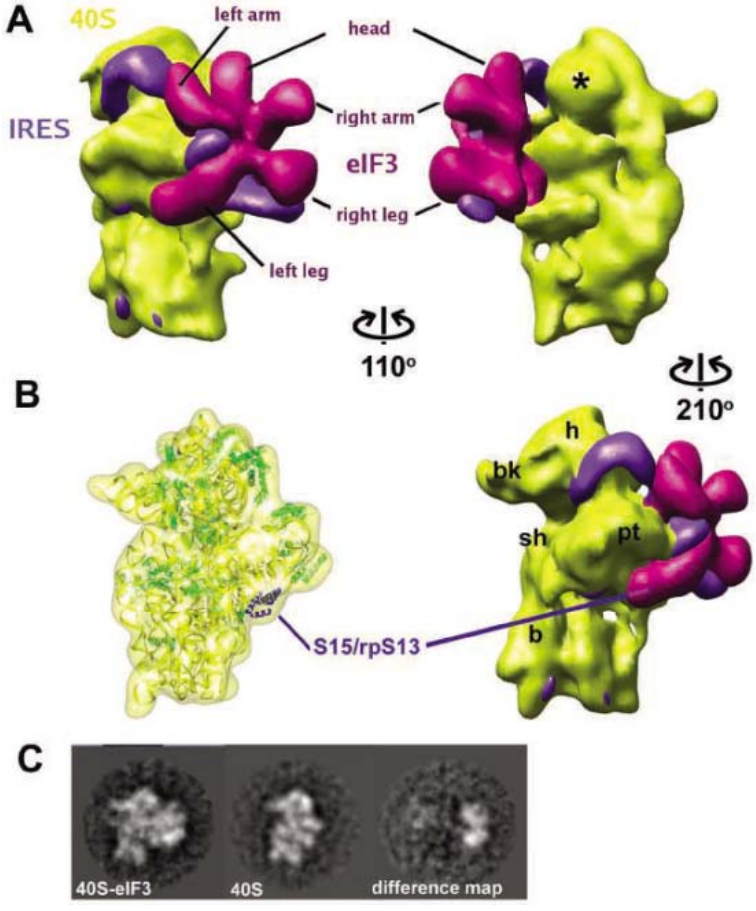

Fig. 3. 40*S*-eIF3-IRES model. (**A**) Views of the 40*S*-eIF3-IRES model; location of RACK1 is indicated by an asterisk. (**B**) 30*S* crystal structure from *T. thermophilus* (*31*) (left, hybrid model) and equivalent view of 40*S*-eIF3-IRES model (right) (b, body; bk, beak; h, head; pt, platform; sh, shoulder). (**C**) EM analysis of 40*S* binding to eIF3, showing class averages in the same orientation from particles with and without bound eIF3.

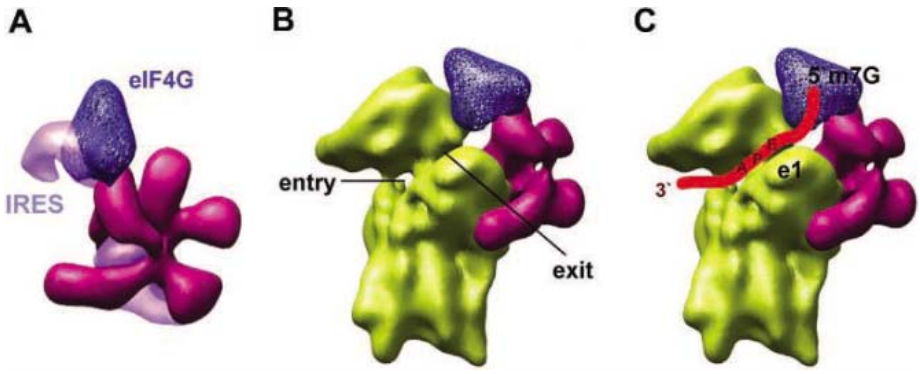

Fig. 4. Mapping of the eIF4G binding site on eIF3 and model of 40*S* complex with eIF3, eIF1, mRNA, and eIF4G. (**A**) Superposition of eIF4G density from difference mapping on the eIF3-IRES. (**B**) Model based on Fig. 4A and Fig. 3. The mRNA entry and exit sites are indicated. (**C**) Model of mRNA loading; e1 indicates eIF1 position (*21*). The A, P, and E sites are indicated. mRNA is depicted as a red line.

thermore, eIF3 may stabilize the binding of other conserved initiation factors that occlude the B2a interface contact, as suggested by a recent cryo-EM reconstruction of a prokaryotic initiation complex (*18*) and biochemical analysis of eukaryotic initiation complex assembly (*19*).

To determine how the HCV IRES might functionally replace eIF4G during translation initiation, we used EM and difference mapping to locate the eIF4G binding site on eIF3. Two-dimensional difference maps generated by comparing class-averages of eIF3-eIF4G and eIF3 alone showed an extra mass located near the eIF3 left arm (fig. S5). The class averages were used for back-projection to reconstruct a low-resolution structure of the complex (*5*). The difference density between the eIF3-eIF4G complex and eIF3 shows extra density located at the tip of the left arm with a mass consistent with the size of eIF4G. Therefore, eIF4G binds to the same arm as that occupied by part of the HCV IRES in the eIF3-IRES complex (Fig. 4A).

The 40*S*-eIF3-IRES model allows us to infer the placement of eIF4G relative to 40*S* and other initiation factors, locating eIF4G very close to the E site (Fig. 4B) (movie S1). This analysis predicts the position of eIF4F and the approximate location of the 5′-m7G cap of an mRNA (Fig. 4C). The placement of the 5′-end of the mRNA near the E site agrees well with the polarity of the mRNA during decoding by the ribosome, in which the 5′-end protrudes from the E site while the 3′-end is pulled into the ribosome toward the decoding site (*20*). Furthermore, in this model, eIF1 (and its bacterial ortholog IF3), the C-terminal domain of which has been mapped to the platform area by chemical probing and cryo-EM, would be located within a reasonable distance to interact with both eIF4G and the left arm of eIF3 (*18*, *21*, *22*). The interaction between *Saccharomyces cerevisiae* eIF4G and eIF1, as well as the interactions between *S. cerevisiae* and mammalian eIF3 and eIF1, have been detected both in vitro and in vivo (*23*, *24*).

The position of eIF4F on eIF3 near the ribosomal E site has implications for both translation regulation and ribosome scanning of the 5′-untranslated region of a nascently bound mRNA during translation initiation (*25*). First, the location of the receptor for activated C-kinase (RACK1) on the head of 40*S* is close to the bound factors (Fig. 3A) (*26*). Because several subunits of eIF3 are phosphorylated in vivo (*27*), our model suggests that RACK1-associated kinases could regulate the function of eIF3 and eIF4F on the ribosome. Phosphorylation of eIF3 by these kinases could be a mode of regulation used during the initiation process. Second, the location of eIF4G near the E site implies that eIF4A, a subunit of eIF4F, binds near the lagging rather than the leading side of the bound mRNA. To initiate protein synthesis, 40*S* "scans" from the 5′-cap structure toward the start codon, requiring energy (in the form of adenosine triphosphate) which is most likely used by eIF4A, a canonical DEX/H-D helicase (*28*). Translation initiation is enhanced by eIF4A even in the absence of secondary structure in the 5′-untranslated region of the mRNA (*29*), and the ribosome itself has RNA unwinding activity (*30*). Therefore, placement of eIF4A near the E site suggests the interesting possibility that eIF4A may establish directionality by preventing the backward movement of the 40*S* ribosomal subunit as it moves toward the start codon.

Comparison of the 40*S*-eIF3-IRES and 40*S*-eIF3-eIF4G models suggests an intriguing similarity in the function of the HCV IRES and eIF4F to anchor the attached mRNA strand, in either case, near the exit site on the head of 40*S*. Such structurally analogous positioning implies mechanistic overlap of HCV IRES and eIF4F activities, possibly explaining why HCV does not need eIF4F for viral protein synthesis (*8*). Ribosome recruitment to an mRNA, a necessary first step in the protein biosynthetic process, thus appears to involve conserved interactions coordinated by eIF3 that correctly load the mRNA into the ribosomal decoding center and prepare 40*S* for assembly into active ribosomes.

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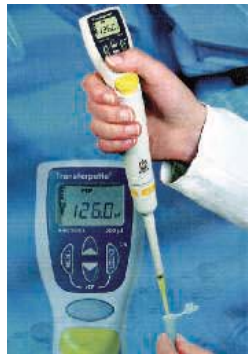

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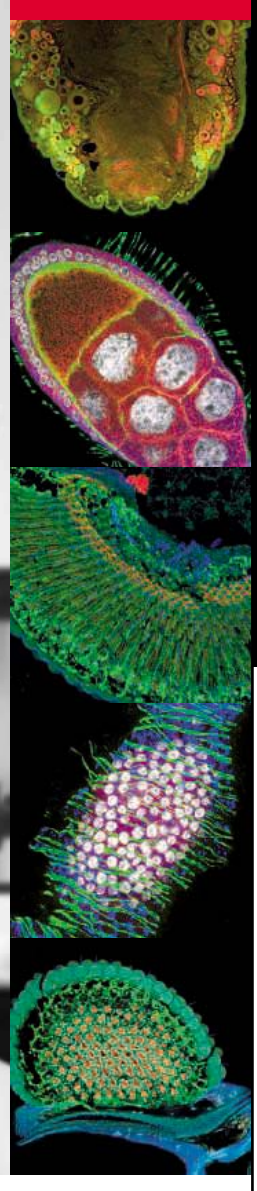

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From Media to Microarrays Cell biologists rely on a variety of physical and chemical tools and technologies, from microscopy to microarrays, in their efforts to understand the fundamental nature of cells and to apply that knowledge for the common good. BY PETER GWYNNE AND GARY HEEBNER

Cell biologists study the physiological properties, behavior, interactions, and environment of cells, at the microscopic and molecular levels. That broad job description covers a wide variety of individual activities. "For basic researchers, the key areas are the very fundamental processes that cells undergo. They study adhesion, differentiation, apoptosis, migration, and many specialized functions of specific cell types," says Laurel Donahue, manager of technical development at **Sigma-Aldrich**. "Industrial cell biologists have narrower focus. They look at the underlying mechanisms that improve the longevity of cells in culture, as well as other processes that will improve protein production. They look at processes that will produce consistent cultures of cells at large scale, and they focus a lot on cell based assays for identifying targets and screening compounds for pharmacological activity."

Whether they work in research or development, most modern cell biologists have one key goal in mind. "The main theme is gaining a better understanding of diseases, through good statistical correlation of molecular interactions with phenotypes," says Ulrich Simon, head of the microscopy business group at **Carl Zeiss**. "We try to shine more light into understanding the root causes of diseases."

That effort divides naturally into specific project areas. For example, the diversity of signal transduction pathways has recently emerged as a critical focus for researchers. "When cells interact with the extracellular milieu, they have to be able to activate the pathways," says Erik Schaefer, vice president for R&D in the signal transduction area at **BioSource International**, a company recently acquired by **Invitrogen**. "Understanding that is the area of functional genomics and proteomics."

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Stem Cells and Beyond

Jörg Pochert, director of the pharma and biotech group at **Hamilton Life Science Robotics**, highlights another area of frantic activity: stem cells. "Scientists face issues of how to do stem cell culturing right," he says. "Also related to stem cells are the questions of what kinds of differentiation protocols are available and how good and stable they are, to ensure that scientists can start a stem cell line and differentiate it into the kind of tissue they need."

To achieve their goals, cell biologists use a mixture of traditional and new tools and technologies, from cell cultures to microscopes to protein microarrays. "Recent techniques include higher resolution mass spectrometry and developments involving very good antibodies," says Mike Schutkowski, vice president of R&D and operations for **JPT Peptide Technologies**. Donahue points out other approaches that have emerged in cell biology labs. "Short interfering RNA continues to be a very important tool," she says. "In addition, information from genomic and proteomic profiling is leading to more hypotheses that can be tested. Industrially, in silico modeling from such companies as **Genomatica** allows for predictive biology. And on the horizon is nanotechnology."

Laboratory automation has also found a critical role in the cell biology lab. "Every major MORE ›››

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pharma and all the major biotechnology companies have a great deal of automation in both their discovery and manufacturing sites," explains Doug Gurevitch, a senior development engineer at the **University of California, San Diego** who is program chair for the **Association for Laboratory Automation**.

Whatever tools they use, cell biologists face a critical problem common to many other life scientists. "The key factor we see over and over again is trying to bring together the data from all the tools out there," explains Brendan Yee, strategic product manager at **Beckman Coulter**. "Cell biologists, especially in drug development, are running into a roadblock created by the amount of data they collect."

The effort to overcome that and other problems in pursuing cell biology involves R&D scientists in academe and industry. "I'm involved in the Cell Migration Consortium," Schaefer says. "This has multimillion dollar projects aimed at bringing together large groups of scientists and making discoveries commercially available. We're working with the consortium to commercialize tools."

Medieval Methodologies

The study of any living cell starts with the use of cell culture media and reagents. "Cell culture media, sera, and related reagents are critically important – more than even investigators who have done cell culture for a long time think," Donahue says. "Culture systems are downright medieval at times." To bring cell cultures into the 21st century and keep cells alive and well during in vitro experiments, researchers and manufacturers have developed a range of growth media, some of which contain only well-defined or synthetic components. Scientists can supplement these "defined" or "serum-free" media with growth factors to study the living cells' responses to changing environments.

Companies such as **ATCC**, **Cambrex**, Invitrogen, and Sigma-Aldrich offer cell culture media, sera, and reagents to grow cells ranging from HeLa to embryonic stem cells. "We look at systems that are being utilized by researchers and try to create products and culture systems that are, for example, serum-free or better defined," Donahue says. "For our industrial customers, who have regulatory constraints, we focus on the development of animal derived component–free media." Recent products from Sigma-Aldrich include EX–CELL Vero, a medium for cells used in research on or production of viral vaccines and two kits that permit industrial researchers to optimize the media for their protein expressing CHO cell lines.

Automation can play a particularly effective role in cell cultures. "Bulk cell culture has been automated for years," Gurevitch explains. "We've been pushing newer work with microcultures for quick, tailored cell development for research, development, and eventually tissue engineering."

Hamilton recently created what it calls its MICROLAB STAR-based CellHost System. Developed in conjunction with Oliver Bruestle of Germany's **University of Bonn**, this automates several aspects of stem cell culture, including media exchange, cell harvesting after trypsinization, plating cells, and the addition of growth factor or other substances to the cell cultures. "The system offers full weekend walk-away times for lab personnel," Pochert says. "It ensures that all the cells are treated homogeneously and that the biological bandwidth of cells you are culturing is narrower than if you use manual methods. We have used it for mouse stem cells, on which we have published two papers."

All About Automation

A special supplement in the 13 January 2006 issue of *Science* will focus on laboratory automation. The supplement will examine selected tools for lab automation and will outline how these devices improve the efficiency of life science research and drug development efforts.

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The Importance of Imaging

Whenever possible, cell biologists want their experiments to show rather than tell. Tools developed in recent years have facilitated that. One area of novel technology overcomes the inherent limitation that cells are colorless and translucent, and hence almost invisible under the lens of a standard light microscope. Biological stains and dyes enable microscopists to look at different cell types and the structures within these cells. "We want to use dyes to indicate how cells interact," Carl Zeiss's Simon explains. Companies such as **Fisher Scientific**, **USB Corporation**, and **Wako Chemicals** offer several stains and dyes, many of them highly purified and use-tested to ensure consistent performance in the laboratory.

Other methods for improving the visibility of cells complement microscopy methods. "Spectral deconvolution techniques introduced in confocal and microscopy techniques put the color issue under control," Simon says. "It has added value to live imaging." Zeiss has recently introduced LSM5 Live, a laser scanning microscope developed in collaboration with Scott Fraser of the **California Institute of Technology**. "This speeds up visualization by a factor of 20 to 30," Simon says. "That opens up new dimensions on research; you can understand color and you're capable of really understanding how proteins interact." In addition to Zeiss, major producers of microscopes include **Leica**, **Nikon Instruments**, and **Olympus**. Beyond refining microscopes, those vendors have developed digital camera systems and software for data analysis.

Apogee/Biodimensions, meanwhile, has developed two noninvasive technologies for in vivo functional imaging at the molecular level. One monitors epithelial inflammation and the other recognizes concentrations of analytes that indicate disease or treatment status. The technology relies on the company's Single System Image (SSI) high performance computing cluster. It permits scientists to compare results from image processing algorithms and photon simulation codes, and thereby evaluate the performances of the approaches during development and testing.

Assays and Antibodies

Imaging doesn't work in isolation. Cell scientists also rely on biochemical assays to determine cellular function. Well-studied subjects of assaying include apoptosis, the cell's cytoskeleton and extracellular matrix, protein phosphorylation, signal transduction pathways, ion channels, nitric oxide, and cell stress. Early entrants into assaying for cell biology include **Alexis Biochemicals**, BioSource, and **Tocris Cookson**. Apoptosis, or programmed cell death, has particular traction. Those com- MORE ›››

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CTACK/CCL27
CTGF
CTGFL/WISP-2
CTLA-4/Fc
CXCL16
E-selectin
EGF
Elafin/SKALP
EMAP-II
ENA-78
Endostatin
Enteropeptidase
Eotaxin
Eotaxin-2
Eotaxin-3 (TSC)
Erk-2
Erythropoietin (EPO)
Exodus-2
Fas Ligand
Fas Receptor
FGF-acidic
FGF-basic
FGF-4
FGF-5
FGF-6
FGF-7/KGF
FGF-8
FGF-9
FGF-10
FGF-16
FGF-17
FGF-18
FGF-19
FGF-20
sFGFR-1 (IIIc)/Fc Chimera
sFGFR-2 (IIIc)/Fc Chimera
sFGFR-3/Fc Chimera
sFGFR-4/Fc Chimera
sFlt-1 (native)
sFlt-1 (D3)
sFlt-1 (D4)
sFlt-1 (D5)
sFlt (D7)/Fc
Flt3-Ligand
sFlt-4
sFlt-4/Fc Chimera
Follicle Stimulating Hormone
Fractalkine/CX3C
G-CSF
PEG-G-CSF
Galectin-1
Galectin-3
Gastrointestinal Cancer Antigen
GCP-2
GDF-3
GDNF
GM-CSF
GRO-α
GRO-β
GRO-γ
GRO/MGSA
Growth Hormone
Growth Hormone 20K
Growth Hormone (Placental)
Growth Hormone BP
GH Releasing Hormone
HCC-1
HGF
HRG1-β1
I-309
I-TAC
IFN-α
IFN-α 1
IFN-α 2
IFN-α 2a
PEG-IFN-α 2a
IFN-α 2b
PEG-IFN-α 2b
IFN-α 4a
IFN-α 4b
IFN-α A
IFN-α B2
IFN-α C
IFN-α D
IFN-α F
IFN-α G
IFN-α H2
IFN-α I
IFN-α J1
IFN-α K
IFN-α WA
IFN-β
IFN-β 1a
IFN-β 1b
IFN-γ
IFN-γ sR Chain 1
IFN-κ
IFN-λ 2
IFN-ω
Leukocyte IFN
IGF-I
Long R^3 IGF-I
IGF-II
proIGF-II
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
IGFBP-7
IκBα
IL-1α
IL-1β
IL-2
sIL-2 Receptor-α
IL-3
IL-4
sIL-4 Receptor
IL-5
IL-6
sIL-6 Receptor
IL-7
IL-8 (72 a.a.)
IL-8 (77 a.a.)
IL-9
IL-10
IL-11
IL-12
IL-12p40
IL-13
IL-13 analog
IL-15
IL-16 (121 a.a.)
IL-16 (130 a.a.)
IL-17
IL-17B
IL-17D
IL-17E
IL-17F
IL-19
IL-20
IL-21
IL-22
Insulin
IP-10
JNK2α1
JNK2α2
sKDR (D7)
sKDR/Fc Chimera
KGF
LAG-1
LALF Peptide
LBP
LBP Natural, Purified
LBP Peptide
LD-78β
LEC/NCC-4
Leptin
LeukinFeron
Leuprolide
Leutenizing Hormone Releasing Hormone
LIF
LIGHT
sLYVE-1
Lymphotactin
M-CSF
MCP-1 (MCAF)
MCP-2
MCP-3
MCP-4
MDC (67 a.a.)
MDC (69 a.a.)
MEC
Mek-1
Menopausal Gonadotrophin
Midkine
MIG
MIP-1α
MIP-1β
Viral MIP-2
MIP-3
MIP-3α
MIP-3β
MIP-4 (PARC)
MIP-5
Myostatin (GDF-8)
Myostatin-Propeptide
NAP-2
Neurturin
beta-NGF
NFAT-1
NOGGIN
NP-1
NT-1/BCSF-3
NT-3
NT-4
Oncostatin M
Osteoprotegerin (OPG)
Ovarian Cancer Antigen
p38-α
PDGF-AA
PDGF-AB
PDGF-BB
Persephin
PF-4
PIGF-1
PIGF-1/His
PIGF-2
Pleiotrophin
PLGF-1
Polymyxin B (PMB)
PRAS40
Prokineticin-2
Prolactin
PTHrP
sRANK
sRANKL
RANTES
RELM-β
Resistin
apo-SAA
SCF
SCF/C-kit Ligand
SCGF-α
SCGF-β
SDF-1α
SDF-1β
SHH
c-Src
STAT1
TACI
TARC
TECK
TFF2
TGF-α
TGF-β1
TGF-β2
TGF-β3
Thymosin α1
sTIE-1/Fc Chimera
TL-1A
TNF-α
TNF-β
sTNF-receptor Type I
sTNF-receptor Type II
TPO
TRAIL/Apo2L
sTRAIL R-1 (DR4)
sTRAIL R-2 (DR5)
Tumor Suppressor p53
TWEAK
TWEAK Receptor
Urokinase
EG-VEGF
VEGF121
VEGF165
Orf Virus VEGF-E
Orf Virus HB-VEGF-E
WISP-1
WISP-2
WNT-1

Mouse Proteins

Acrp30
April
BLC/BCA-1
C-10
Cardiotrophin-1
CD14
sCD40 Ligand/TRAP
CD105/Endoglin
CTACK/CCL27
CXCL16
EGF
Eotaxin
Eotaxin-2
Exodus-2
FGF-9
FGF-basic
Flt3-Ligand
G-CSF
GM-CSF
GRO-β/MIP-2
GRO/KC/CINC-1
I-TAC
IFN-α
IFN-α A
IFN-β
IFN-γ
IFN-γ sR chain 1
IFN-λ 2
IGF-I
IGFBP-5
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-6
IL-7
IL-9
IL-10
IL-12
IL-12p40
IL-13
IL-15
IL-17
IL-20
IL-22
IP-10
I-TAC
JE (MCP-1)
KC
LBP
Leptin
LIGHT
Limitin
LIX
sLYVE-1
M-CSF
MCP-2
MCP-3
MCP-5
MDC
MEC
MIG
MIP-1 α
MIP-1 β
MIP-1 γ
MIP-2
MIP-3 α
MIP-3 β
NGF
sRANKL
RANTES
RELM-α
RELM-β
Resistin
SCF
SDF-1α
SDF-1β
SF20
TNF-alpha
TPO
VEGF164

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» advances in: Cell Biology

panies and others such as **EMD Biosciences**, **Roche Applied Science**, and **Trevigen** offer assays for studying it.

Antibodies represent major research tools for cell biology and signal transduction. For example, a key development in the study of kinases – molecules that control many cellular functions – was the production of specific targeted antibodies that identify the active form of protein kinases. Those tools improved researchers' understanding of the role of kinases in various signal transduction pathways. Companies such as BioSource, **BD Biosciences Pharmingen**, **Chemicon**, and **Upstate** offer antibodies to study the role of kinases in signal transduction.

BioSource has another focus. "One of the things we've done with our antibodies is put them into platforms to understand the kinetics and overall stoichiometry of phosphorylation events," Schaefer explains. "We have patent-pending technology to detect phosphorylation for certain protein events and to work out how much is phosphorylated. We also have a phosphoarray chip platform. And we offer beads that allow you to get up to 100 combinations and dye signatures to capture proteins of interest, and identify them with a detection antibody."

In September, Beckman Coulter introduced a custom kit to measure ZAP-70 protein expression in whole blood specimens using flow cytometry. ZAP-70 (Zeta-associated protein, 70 kiloDaltons) is an intracellular protein associated with signal transduction networks in lymphocyte populations whose expression has been associated with disease progression in a group of patients with chronic lymphocytic leukemia, the most common leukemia in the United States and Europe. "There's interest in whether ZAP-70 protein expression marks a group of patients in whom the disease will progress rapidly," explains Vincent Shankey, a senior staff development scientist at Beckman Coulter's Advanced Technology Center. "Our kit contains an optimized antibody to ZAP-70, a unique fixation and permeabilization technique to monitor intracellular signal transduction protein epitopes, a gating and analysis technique to measure ZAP-70 protein expression in key cell populations within the sample, and a rigorous protocol for researchers to follow to set up a flow cytometer to measure the protein."

Microarraying Methods

The introduction of DNA microarrays quickly sparked the idea of using this miniaturized platform with other biomolecules, such as proteins and peptides. **Biacore** and **Ciphergen** have developed proprietary array systems for analyzing proteins. And JPT Peptide Technologies has developed its own approach. "We were first on the market with peptide arrays in 2000," Schutkowski says. "We have technology that synthesizes thousands of peptides per week."

The firm's unique peptide microarrays accelerate crucial steps in drug discovery programs by focusing on enzyme families such as kinases and proteases. The company's pepSTAR platform identifies substrates in less than one week and profiles kinases and proteases. JPT also customizes peptide chips to target specific classes of enzymes. And it provides an all-in-one service that includes screening microarrays, evaluating data, and synthesizing, characterizing, and optimizing selected substrates. "The logical extension of these products," Schutkowski explains, "is to use the microarrays with samples from human patients – looking for antibodies against HIV, for example."

Advances in cell biology hold great promise for basic scientific understanding and the treatment of disease. The tools and techniques in application and under development offer the promise of discoveries that will add to scientists' understanding of how cells function and the effective application of that knowledge.

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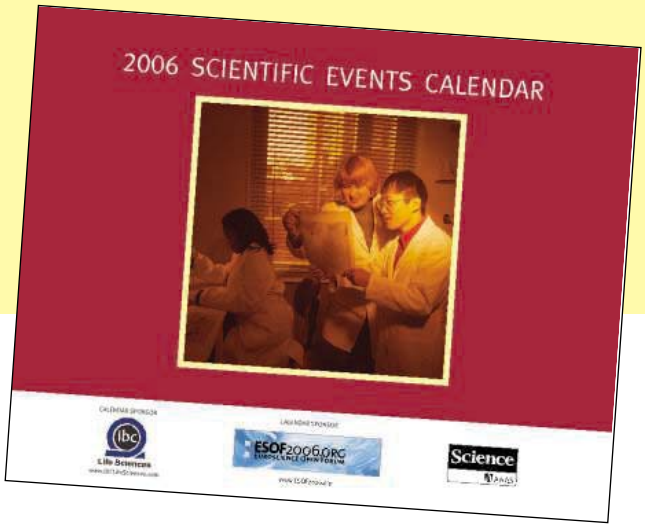
Events of 2006

A calendar of meetings, conferences, and other events for the coming year



AAAS creates and sponsors a wide range of events every year to support science. The most elaborate is the annual meeting. This year's meeting, "Grand Challenges, Great Opportunities," will take place on 16–20 February 2006 in St. Louis—"The Biobelt," home to 390 plant and life science research centers. "This is a major area for development of research and technology," says AAAS President Gilbert S. Omenn.

With 196 symposia, this year's meeting will surpass those of the past. Special events include a day-long program, "Beyond Pi: Grand Challenges in Mathematical Sciences" and a Sunday afternoon program on "The Teaching of Evolution" for educators, annual meeting registrants, and invited guests. Moreover, the meeting caters to more than scientists. Family Science Days will include a zoo display, a tractor-trailer exhibit from Monsanto, a garden store from the Missouri Botanical Garden, and simulations of hurricanes and storms. In the past, more than 3,000 people turned out for the family event, which is designed for parents, students, and science teachers, for whom there will be lesson plans.

The annual meeting will also have a wide variety of talks. Omenn will give the AAAS Presidential Address. Major lecturers include Nobel Laureate Peter Agre from the Duke University Medical Center on water channels in living systems, Pamela Matson from Stanford University on ecosystem sustainability, Mario Capecchi of the University of Utah School of Medicine on gene targeting against human disease, and Robert Fraley of Monsanto talking about biotechnology for crops.

The events of this year, however, go far beyond the annual meeting. In 2006, one of the biggest campaigns celebrates the merging of ScienceCareers.org and *Science*'s Next Wave onto one website. *Science*'s Next Wave and ScienceCareers.org organize and run programs throughout the year and around the world. Garth Fowler, senior program associate for Next Wave and ScienceCareers.org, says, "We focus on trainees, graduate students, postdocs, and scientists who just started their careers"—developing seminars, websites, and partnerships with career centers. One upcoming program, "Interviewing Skills for Scientists," will include "two parallel tracks: the interview process for jobs in industrial research and for becoming a professor or lecturer," according to Fowler. He adds, "Through the Next Wave and ScienceCareers.org website, we keep our finger on the pulse of what people are looking for, and then orient our workshops and panels to meet those needs."

Fowler says, "We are going to large conferences, like the Society for Neuroscience, showing the new site and how to work through it." In addition, Fowler and his colleagues are putting together a scientific career development course. "This two- to three-day course," says Fowler, "gives postdocs and young faculty the skills that they do not get taught: how to manage a lab full of postdocs, technicians, and so on." He adds, "We will also incorporate ideas that postdocs and young faculty said they were interested in." This course will be taken on the road to universities and conferences.

Next Wave also provides support outside the United States. Seema Sharma, European program director, says, "We plan workshops for Ph.D. graduate students and postdocs with hands-on, practical information to advance their careers." This includes writing grant applications, working in industry as a career, and moving from academia to a nonresearch career. She adds, "A big issue that we have is mobility around Europe—where to get funding for mobility opportunities." Sharma and her colleagues also work on gender issues in their events. She says, "We try to address the gender imbalance in science and see what can get women back into science after having children and a career break." During the past year, Sharma and her team traveled to Bulgaria, Cambridge in the U.K., France, Ireland, Sweden, Poland, and other locations.

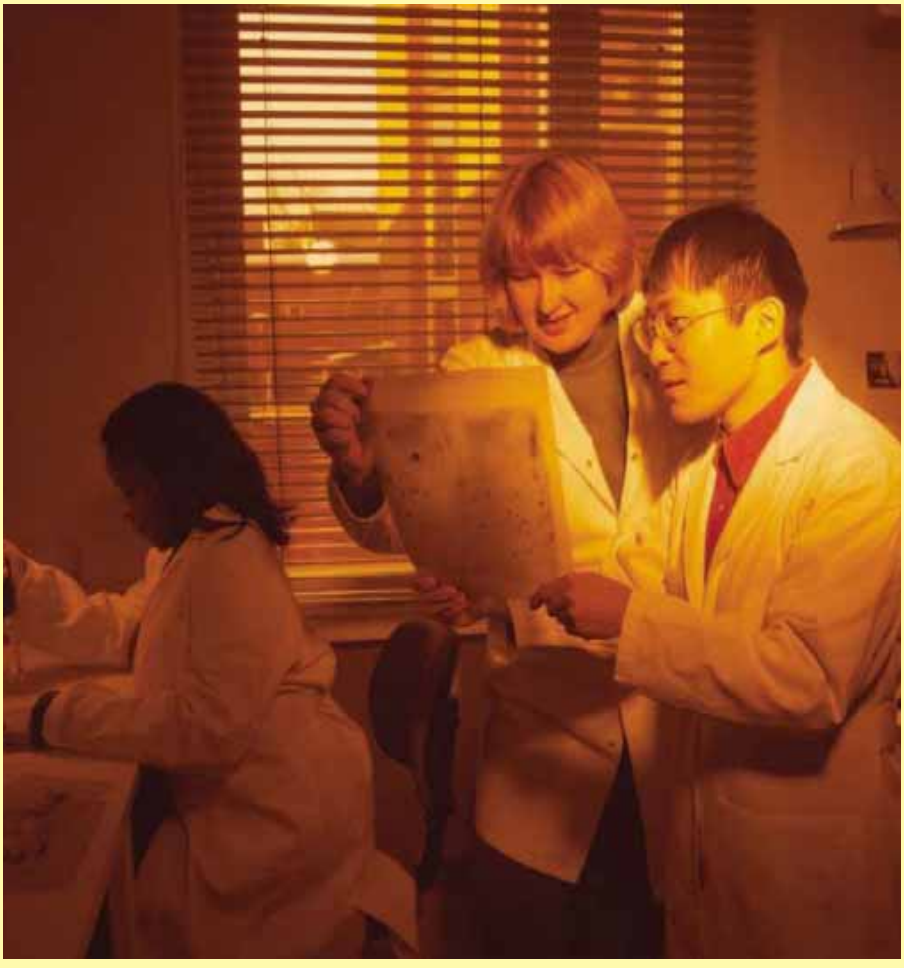
As the following pullout calendar shows, many more events lie ahead. It will be an exciting year of programs and information. **MIKE MAY**

PREPARING FOR 2007

Do you have responsibility for a meeting or other event in 2007 that you would like included in next year's calendar? You can find out how to submit your listing at http://www.sciencemeetings.org

You can check out details of the 2006 AAAS Annual Meeting at http://www.aaas.org/meetings/Annual_Meeting/

2006 SCIENTIFIC EVENTS CALENDAR



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Science
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Science 2006 Scientific Events Calendar

Date	Event	Location
January		
8 – 12 Jan	American Astronomical Society 207th Meeting	Washington, DC, USA
21 – 25 Jan	Lab Automation 2006	Palm Springs, CA, USA
21 – 25 Jan	AAPT National Meeting	Anchorage, AK, USA
February		
2 – 4 Feb	5th International from Gene to Cure Conference—Breast Cancer: From Gene to Cure	Amsterdam, Netherland
5 – 10 Feb	Ultrafast Phenomena in Cooperative Systems	Buellton, CA,USA
7 – 10 Feb	Management of Vector-Borne Viruses	Hyderabad, India
8 – 11 Feb	BIFI 2006 - II—International Conference from Physics to Biology: The Interface between Experiment and Computation	Zaragoza, Spain
9 – 11 Feb	3rd International Conference Ubiquitin, Ubiquitin-like Proteins, and Cancer	Houston, TX, USA
9 – 10 Feb	Politics and Science: How Their Interplay Results in Public Policy	New York, NY, USA
12 – 17 Feb	Genes and Behavior	Ventura, CA, USA
13 – 15 Feb	Drug Development Summit	Phoenix, AZ, USA
15 – 17 Feb	Conference on Data Mining, Systems Analysis, and Optimization in Neuroscience	Gainesville, FL, USA
16 – 18 Feb	Cancer Prevention 2006	St. Gallen, Switzerland
16 – 18 Feb	International Stroke Conference 2006	Kissimmee, FL, USA
16 – 20 Feb	AAAS Annual Meeting	St. Louis, MO, USA
27 – 28 Feb	9th Annual Process Validation	Carlsbad, CA,USA
March		
1 – 3 Mar	16th Annual Antibody Production and Downstream Processing	Carlsbad, CA, USA
1 – 2 Mar	A Workshop for Early Career Researchers	Madrid, Spain
3 – 6 Mar	2nd International Meeting of Physiology and Pharmacology of Temperature Regulation	Phoenix, Arizona, USA
5 – 10 Mar	New Antibacterial Discovery and Development	Ventura, CA, USA
7 – 11 Mar	2006 Annual Meeting, Association of American Geographers	Chicago, IL, USA

	For Information	Event Host
	http://www.aas.org	American Astronomical Society
	http://labautomation.org	Association for Laboratory Automation
	http://www.aapt.org	American Association of Physics Teachers
s	http://www.eurcancen.org/genetocure06/index.htm	European Cancer Centre
	http://www.grc.org/programs/2006/ultra.htm	Gordon Research Conferences
	http://www.mvbv2006.org	International Crop Institute for the Semi-Arid Tropics
	http://bifi.unizar.es/events/bifi2006/index.html	Institute for Biocomputation and Physics of Complex Systems
	http://www.sentrin.org	The University of Texas M. D. Anderson Cancer Center
	http://www.socres.org	New School for Social Research
	http://www.grc.org/programs/2006/genes.htm	Gordon Research Conferences
	http:ugdevelopmentsummit.com	Engel Conferences
	http://www.ise.ufl.edu/cao/neuroscience2006/	University of Florida
	http://www.oncoconferences.ch	St. Gallen Oncology Conferences
	http://www.strokeconference.org	American Stroke Association
	http://www.aaasmeetings.org	American Association for the Advancement of Science
	http://www.IBCLifeSciences.com	IBC Life Sciences
	http://www.IBCLifeSciences.com/antibodyprod	IBC Life Sciences
	http://sciencecareers.sciencemag.org/meetings/career_development_workshop_for_researchers	*Science*'s Next Wave and Federacion de Jovenes Investigadores
	http//www.FeverLab.net/meeting	Thermal Section, International Union of Physiological Sciences
	http://www.grc.org/programs/2006/antibact.htm	Gordon Research Conferences
	http://www.aag.org/annualmeetings	AAG

Data compiled by the *Science* Office of Publishing and Member Services

Science 2006 Scientific Events Calendar

Date	Event	Location
9 – 12 Mar	Neuronal Circuits: From Structure to Function	Cold Spring Harbor, NY
11 – 15 Mar	37th Annual Meeting, American Society for Neurochemistry	Portland, OR, USA
11 – 14 Mar	Memory and Brain: Basic Mechanisms and Clinical Applications	Irvine, CA, USA
12 – 17 Mar	Fibroblast Growth Factors in Development and Disease	Ventura, CA, USA
12 – 17 Mar	Viral Vectors for Gene Therapy, The Science of Clinical Applications	Ventura, CA, USA
13 – 16 Mar	16th Annual AEHS Meeting and West Coast Conference	San Diego, CA, USA
13 – 14 Mar	5th Annual North America Forum for Investing and Partnering in Biotech	Boston, MA, USA
14 – 15 Mar	Chemicals: L-Arginine	Kiev, Ukraine
14 – 15 Mar	44th Robert H. Goddard Memorial Symposium	Greenbelt, MD, USA
15 – 18 Mar	40th Annual Scientific Meeting, ESCI	Prague, Czech Republic
15 – 19 Mar	European Fission Yeast Meeting	Hinxton, Cambridge, UK
15 – 19 Mar	PTEN Pathways	Cold Spring Harbor, NY
16 – 18 Mar	4th International Symposium on Targeted Anticancer Agents	Amsterdam, Netherland
16 – 22 Mar	TALENTE 2006 -— International Competition for Young Makers, Designers, and Technicians	Munich, Germany
19 – 22 Mar	Annual Conference of the Association for General and Applied Microbioogy	Jena, Germany
20 – 22 Mar	R&D Leaders' Forum, Spring 2006	Coral Gables, FL, USA
20 – 21 Mar	2nd National Conference on Obesity and Health	Manchester, UK
22 – 24 Mar	ScreenTech® 2006: Where Technologies and Innovation Meet to Expedite Applications	San Diego, CA, USA
23 – 26 Mar	Systems Biology: Global Regulation of Gene Expression	Cold Spring Harbor, NY
25 – 29 Mar	AMI 2006 Annual Conference and Exhibition	Orlando, FL, USA
29 Mar – 2 Apr	Integrated Biology of Crop Plants	Hinxton, Cambridge, UK
30 Mar – 1 Apr	1st Amsterdam Diabetes Forum	Amsterdam, Netherland
April		
1 – 5 Apr	ECE 2006: 8th European Congress of Endocrinology	Glasgow, UK
1 – 5 Apr	ASPET Annual Meeting at Experimental Biology 2006	San Francisco, CA, USA
3 – 11 Apr	Microarrays and the Transcriptome	Cambridge, UK
5 – 8 Apr	21st European Association of Urology Annual Congress	Paris, France
6 Apr	UCSF Career Fair	San Francisco, CA, USA
9 – 12 Apr	BIO 2006	Chicago, IL, USA

	For Information	Event Host
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.ASNeurochem.org	ASN
	http://www.cnlm.uci.edu	Center for the Neurobiology of Learning and Memory, UC Irvine
	http://www.grc.org/programs/2006/fibro.htm	Gordon Research Conferences
	http://www.grc.org/programs/2006/viral.htm	Gordon Research Conferences
	http://www.aehs.com/conferences/westcoast/index.htm	Association for Environmental Health and Sciences
	http://www.sachsforum.com	Sachs Associates
	http://www.arginine.i8.com	National Bio Science
	http://www.astronautical.org	American Astronautical Society
	http://www.esci.eu.com	European Society for Clinical Investigation
	http://www.wellcome.ac.uk/conferences	The Wellcome Trust
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
s	http://www.nddo.org	NDDO Research Foundation and ESMO
	http://www.hwk-muenchen.handwerk.de/webview/view?pnr=447&onr=74	International Trade Fair for Small and Medium Sized Enterprises
	http://www.vaam.de	The Association for General and Applied Microbiology
	http://www.phacilitate.co.uk/leaders	Phacilitate
	http://www.obesityandhealth.co.uk	Conference Secretariat
	http://www.IBCLifeSciences.com/ScreenTech	IBC Life Sciences
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.ami-imaging.org	Academy of Molecular Imaging
	http://www.wellcome.ac.uk/conferences	The Wellcome Trust / European Science Foundation
s	http://www.marktwo.nl/diabetesforum	Amsterdam RAi
	http://www.euro-endo.org/conferences/congress.htm	Society for Endocrinology
	http://www.faseb.org/meetings/eb2006	American Society for Pharmacology and Experimental Therapeutics
	http://www.wellcome.ac.uk/doc_wtx026218.html	The Wellcome Trust
	http://www.eauparis2006.org/	European Association of Urology
	http://saawww.ucsf.edu/career	University of California, San Francisco
	http://www.bio.org	Biotechnology Industry Organization

Science 2006 Scientific Events Calendar

Date	Event	Location
17 – 21 Apr	2006 MRS Spring Meeting	San Francisco, CA , US
18 – 22 Apr	Channels, Receptors, and Synapses	Cold Spring Harbor, NY
20 – 21 Apr	AAAS Forum on Science and Technology Policy	Washington, DC, USA
21 – 22 Apr	1st European Conference on Scientific Publishing in Biomedicine and Medicine	Lund, Sweden
25 – 30 Apr	2006 World DNA and Genome Day	Dalian, China
26 – 30 Apr	Gene Expression and Signaling in the Immune System	Cold Spring Harbor, NY
27 Apr – 2 May	Frontiers in Myogenesis	Callaway Gardens, GA,
27 – 28 Apr	Annual Biodiversity Symposium: Conserving Birds in Human-Dominated Landscapes	New York City, NY, USA
May		
1 – 4 May	TIDES® 2006: Oligonucleotide and Peptide Technology and Product Development Conference	Carlsbad, CA, USA
1 – 3 May	RNAi-Boston-2006 Meeting on RNA Interference: Biochemical Genetics to Drugs and Therapeutics	Boston-Waltham, MA, U
3 – 6 May	9th Congress of the European Society of Contraception: Improving Life Quality through Contraception and Reproductive Health Care	Istanbul, Turkey
3 – 7 May	Molecular Chaperones and the Heat Shock Response	Cold Spring Harbor, NY
8 – 10 May	9th Annual Conference on Vaccine Research	Baltimore, MD, USA
8 – 11 May	Nanotrends 2006	Potsdam, Germany
8 – 10 May	Open Door Workshop: Working with the Human Genome Sequence	Cambridge, UK
9 – 11 May	Workshop on Clustering Problems in Biological Networks	Piscataway, NJ, USA
10 – 14 May	The Biology of Genomes	Cold Spring Harbor, NY
10 – 13 May	CSNA Annual Meeting	Piscataway, NJ, USA
14 – 17 May	SCIpharm 2006	Edinburgh, UK
14 – 20 May	Molecular Basis of Infection: Basic and Applied Research Approaches	Cambridge, UK
17 – 21 May	The Cell Cycle	Cold Spring Harbor, NY
23 – 28 May	Retroviruses	Cold Spring Harbor, NY
28 May – 1 June	3rd EPSO Conference—Plant Dynamics: From Molecules to Ecosystems	Visegrád, Hungary
30 May – 3 June	9th Conference on the Intersections of Nuclear and Particle Physics	Rio Grande, Puerto Ric

Visit www.sciencemeetings.org

	For Information	Event Host
A	http://www.mrs.org	Materials Research Society
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.aaas.org/spp/rd/forum.htm	American Association for the Advancement of Science
	http://www.ecspbiomed.net/	Faculty of Medicine, Lund University
	http://www.dnaday.com	World High Technology Society
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
USA	http://www.pharm.emory.edu/myogenesis2006/	Society for Muscle Biology
	http://cbc.amnh.org/symposia/birds/	American Museum of Natural History, Center for Biodiversity and Conservation
	http://www.IBCLifeSciences.com/TIDES	IBC Life Sciences
SA	http://www.expressgenes.com	GeneExpression Systems, Inc.
	http://www.contraception-esc.com/istanbul.htm	European Society of Contraception
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.nfid.org/conferences	National Foundation for Infectious Diseases
	http://www.nanotrends.de	IIR Deutschland GmbH
	http://www.sanger.ac.uk/Info/workshops/opendoor/	The Wellcome Trust
	http://dimacs.rutgers.edu/Workshops/Clustering/	Center for Discrete Mathematics and Theoretical Computer Science
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.rutgers.edu/Workshops/CSNA	Classification Society of North America
	http://www.scipharm.info	Society of Chemical Industry
	http://www.wellcome.ac.uk/doc_wtx026211.html	The Wellcome Trust
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.epsoweb.org/catalog/conf2006.htm	European Plant Science Organisation
o	http://cipanp.physics.uiuc.edu/	University of Illinois at Urbana-Champaign

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Science 2006 Scientific Events Calendar

Date	Event	Location
30 May – 2 June	33rd Annual International Global Health Conference—Excellence, Innovation, and Influence: Pathways to Results	Washington, DC, USA
31 May – 2 June	14th Annual Meeting, SPR	San Antonio, TX, USA
31 May – 5 June	71st Symposium: Regulatory RNAs	Cold Spring Harbor, NY
31 May – 3 June	HGM 2006	Helsinki, Finland
June		
1 – 5 June	FOCIS 2006	San Francisco, CA, US
3 – 17 June	FASEB Summer Research Conferences	Saxtons River, VT, USA Snowmass Village, CO, Indian Wells, CA, USA Tucson, AZ, USA
4 – 8 June	12th International Symposium on Environmental Analytical Chemistry	Hamburg, Germany
4 – 9 June	Environmental Endocrine Disruptors	Il Ciocco, Barga, Italy
11 – 16 June	Iron-Sulfur Enzymes	New London, NH, USA
11 – 14 June	Working with Pathogen Genomes	Montevideo, Uruguay
11 – 16 June	Physics Research and Education	South Hadley, MA, USA
17 – 21 June	SDB 65th Annual Meeting	Ann Arbor, MI, USA
18 – 23 June	Single Molecule Approaches to Biology	New London, NH, USA
18 – 23 June	Thiol-Based Redox Regulation and Signaling	Biddeford, ME, USA
20 – 25 June	RNA 2006	Seattle, WA, USA
21 – 30 June	Functional Genomics	Cambridge, UK
22 – 25 June	PSI Symposium: Plant Receptor Signaling	Ames, IA, USA
25 – 30 June	MEMS Technology and Biomedical Applications	New London, CT, USA
25 June	SWST 49th Annual Convention	Newport Beach, CA, U
25 – 30 June	Permeable Sediments	Waterville, ME, USA
26 – 29 June	ECM VII: Cartilage and Joint Repair	Davos, Switzerland
29 June – 1 July	ISSCR 4th Annual Meeting	Toronto, Canada
July		
2 – 7 July	International Conference on Porphyrins and Phthalocyanines	Rome, Italy
2 – 7 July	Vibrational Spectroscopy	Biddeford, ME, USA
3 July	Lucerne Fuel Cell Forum 2006	Lucerne, Switzerland
9 – 12 July	ASP 33rd Biennial Meeting	Rio Grande, Puerto Ric

	For Information	Event Host
	http://www.globalhealth.org/conference	Global Health Council
	http://www.preventionresearch.org	Society for Prevention Research
, USA	http://meetings.cshl.edu	Cold Spring Harbor
	http://hgm2006.hugo-international.org/	HUGO, The Human Genome Organisation
A	http://www.focisnet.org	Federation of Clinical Immunology Societies
USA	http://src.faseb.org	FASEB Summer Research Conferences
	http://www.iaeac.ch	International Association of Environmental and Analytical Chemistry
	http://www.grc.org/programs/2006/envendo.htm	Gordon Research Conferences
	http://www.grc.org/programs/2006/iron.htm	Gordon Research Conferences
	http://www.wellcome.ac.uk/doc_wtx027325.html	The Wellcome Trust
	http://www.grc.org/programs/2006/physres.htm	Gordon Research Conferences
	http://www.sdbonline.org	Society for Developmental Biology
	http://www.grc.org/programs/2006/single.htm	Gordon Research Conferences
	http://www.grc.org/programs/2006/thiol.htm	Gordon Research Conferences
	http://www.rnasociety.org/#RNASocEvents	The RNA Society
	http://www.wellcome.ac.uk/doc_wtx026850.html	The Wellcome Trust
	http://www.plantsciences.iastate.edu/symposia/	Plant Sciences Institute, Iowa State University
	http://www.grc.org/programs/2006/mems.htm	Gordon Research Conferences
SA	http://www.swst.org	Society of Wood Science and Technology
	http://www.grc.org/programs/2006/permsed.htm	Gordon Research Conferences
	http://www.ecmjournal.org/ecm_meetings	European Cells and Materials
	http://www.isscr.org/meetings	International Society for Stem Cell Research
	http://icpp.uniroma2.it	University of Rome “Tor Vergata”
	http://www.grc.org/programs/2006/vibrat.htm	Gordon Research Conferences
	http://www.efcf.com	European Fuel Cell Forum
o	http://www.photobiology.org	American Society for Photobiology

Data compiled by the *Science* Office of Publishing and Member Services

Science 2006 Scientific Events Calendar

Date	Event	Location
10 – 28 July	Spinal Cord Injury Research Techniques	Irvine, CA, USA
15 – 19 July	Euroscience Open Forum 2006	Munich, Germany
16 – 22 July	5th International Conference on Bioinformatics of Genome Regulation and Structure (BGRS 2006)	Novosibirsk, Russia
16 – 23 July	36th COSPAR Scientific Assembly and Associated Events	Beijing, China
16 – 21 July	International Biometrics Conference	Montreal, Canada
16 July – 5 Aug	Science Summer School	Cambridge, UK
19 – 25 July	Human Genome Analysis: Genetic Analysis of Multifactorial Diseases	Cambridge, UK
20 – 26 July	Molecular Neurology and Neuropathology	Cambridge, UK
20 – 24 July	Glia in Health and Disease	Cold Spring Harbor, NY
22 – 26 July	ASP Annual Meeting	Crystal City, VA, USA
23 – 27 July	BioScience 2006: Bioscience for the 21st Century	Glasgow, UK
23 – 28 July	Marine Microbes	Biddeford, ME, USA
23 – 28 July	Plasmonics	Keene, NH, USA
August		
6 – 11 Aug	8th International Conference on Mercury as a Global Pollutant	Madison, WI, USA
6 – 11 Aug	Mechanisms of Epilepsy and Neuronal Synchronization	Waterville, ME, USA
6 – 10 Aug	11th International Congress of Human Genetics	Brisbane, Australia
7 – 10 Aug	11th Annual Drug Discovery® and Development World Congress	Boston, MA, USA
9 Aug	*Science* Career Fair	Boston, MA, USA
13 – 18 Aug	Ceramics, Solid State Studies in	Andover, NH, USA
13 – 18 Aug	Drinking Water Disinfection By-Products	South Hadley, MA, USA
13 – 18 Aug	Post-Transcriptional Gene Regulation, The Biology of	Oxford, UK
16 – 20 Aug	Mechanisms and Models of Cancer	Cold Spring Harbor, NY
20 – 25 Aug	Brain Energy Metabolism and Blood Flow	Oxford, UK
23 – 27 Aug	Molecular Genetics of Bacteria and Phages	Cold Spring Harbor, NY
27 Aug – 1 Sept	Biology of 14-3-3 Proteins	Oxford, UK
30 Aug – 2 Sept	Interactome Networks	Cambridge, UK

Visit www.sciencemeetings.org

	For Information	Event Host
	http://www.reeve.uci.edu/	University of California, Irvine
	http://www.esof2006.org	Euroscience Open Forum
	http://www.bionet.nsc.ru/meeting/bgrs2006/	Institute of Cytology and Genetics SB RAS
	http://meetings.copernicus.org/cospar2006/	Committee on Space Research
	http://www.ibc2006.org	International Biometrics Society
	http://www.cont-ed.cam.ac.uk/IntSummer	University of Cambridge
	http://www.wellcome.ac.uk/doc_wtx026851.html	The Wellcome Trust
	http://www.wellcome.ac.uk/advancedcourses	The Wellcome Trust Advanced Courses
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.phcog.org	American Society of Pharmacognosy
	http://www.BioScience2006.org	Biochemical Society
	http://www.grc.org/programs/2006/marinem.htm	Gordon Research Conferences
	http://www.grc.org/programs/2006/plasmon.htm	Gordon Research Conferences
	http://www.mercury2006.org	University of Wisconsin-Madison, U.S. Geological Survey, UW-La Crosse
	http://www.grc.org/programs/2006/epilepsy.htm	Gordon Research Conferences
	http://www.ichg2006.com	Human Genetics Society of Australasia and International Federation of Human Genetics Societies
	http://www.drugdisc.com	IBC Life Sciences
	http://sciencecareers.sciencemag.org/feature/fair/career-fair.shtml	*Science*/ScienceCareers.org
	http://www.grc.org/programs/2006/ceramics.htm	Gordon Research Conferences
	http://www.grc.org/programs/2006/drinking.htm	Gordon Research Conferences
	http://www.grc.org/programs/2006/posttran.htm	Gordon Research Conferences
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.grc.org/programs/2006/brain.htm	Gordon Research Conferences
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.grc.org/programs/2006/14-3-3.htm	Gordon Research Conferences
	http://www.wellcome.ac.uk/conferences	The Wellcome Trust / Cold Spring Harbor Laboratory

Data compiled by the *Science* Office of Publishing and Member Services

Science 2006 Scientific Events Calendar

Date	Event	Location
30 Aug – 3 Sept	Mouse Molecular Genetics	Cold Spring Harbor, NY

September

Date	Event	Location
Sept	High-throughput Approaches to Cancer	Hinxton, Cambridge, UK
3 – 9 Sept	Photon06	Manchester, UK
3 – 8 Sept	Molecular Mechanisms in Lymphatic Function and Disease	Les Diablerets, Switzerl
4 – 8 Sept	21st European Photovoltaic Solar Energy Conference and Exhibition	Dresden, Germany
6 – 10 Sept	Genomic Perspectives on Host Pathogen Interactions	Hinxton, Cambridge, UK
6 – 8 Sept	BioKOREA2006	Seoul, Korea
6 – 10 Sept	Translational Control	Cold Spring Harbor, NY
10 – 13 Sept	5th International Symposium on Hormonal Carcinogenesis	Montpellier, France
13 – 17 Sept	Axon Guidance, Synaptogenesis and Neural Plasticity	Cold Spring Harbor, NY
14 – 16 Sept	3rd International Ph.D. Student Symposium—Horizons in Molecular Biology	Göttingen, Germany
18 – 20 Sept	Open Door Workshop: Working with the Human Genome Sequence	Cambridge, UK
24 – 29 Sept	Computational Aspects—Biomolecular NMR	Aussois, France
25 – 27 Sept	7th International Conference on the Scale Up of Chemical Processes	Vilamoura, Portugal
25 – 28 Sept	13th Annual Chips to Hits® Conference and Exhibition	Boston, MA, USA
27 Sept –1 Oct	Genome Informatics	Hinxton, Cambridge, UK
27 Sept – 1 Oct	Dynamic Organization of Nuclear Function	Cold Spring Harbor, NY
28 Sept – 2 Oct	From Human Genetic Variations to Prediction of Risks and Responses to Drugs and to the Environment	Thira, Greece

October

Date	Event	Location
Oct	Vaccine Adjuvants	Hinxton, Cambridge, UK
1 – 6 Oct	2006 International Conference on Aminoacyl-tRNA Synthetases	San Diego, CA, USA
3 – 7 Oct	Design and Analysis of Genetic-based Association Studies	Cambridge, UK
4 – 5 Oct	Molecular Genetics of Aging	Cold Spring Harbor, NY
4 – 9 Oct	60th Annual Fall Conference and Scientific Sessions of the Council for High Blood Pressure Research in association w/ the Council on the Kidney in CVD	San Antonio, TX, USA

	For Information	Event Host
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
K	http://www.wellcome.ac.uk/conferences	The Wellcome Trust / Cold Spring Harbor Laboratory
	http://www.photon06.org	The Institute of Physics and the UK Consortium for Photonics and Optics
land	http://www.grc.org/programs/2006/lymphat.htm	Gordon Research Conferences
	http://www.photovoltaic-conference.com	WIP Renewable Energies - Munich
K	http://www.wellcome.ac.uk/conferences	The Wellcome Trust / Cold Spring Harbor Laboratory
	http://www.khidi.or.kr	Korea Health Industry Development Institute and KITA
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.kumc.edu/hormonecancers	INSERM and the University of Montpellier
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.horizons.uni-goettingen.de	International Max Planck Research School for Molecular Biology
	http://www.sanger.ac.uk/Info/workshops/opendoor/	Dr. Rebecca Twells
	http://www.grc.org/programs/2006/bio_nmr.htm	Gordon Research Conferences
	http://www.scientificupdate.co.uk	Scientific Update LLP
	http://www.chipstohits.com	IBC Life Sciences
K	http://www.wellcome.ac.uk/conferences	The Wellcome Trust / Cold Spring Harbor Laboratory
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://biol.prospective-conf.u-nancy.fr	Biologie prospective
K	http://www.wellcome.ac.uk/conferences	The Wellcome Trust
	http://www.aars2006.com/	Paul Schimmel, The Scripps Research Institute
	http://www.wellcome.ac.uk/advancedcourses	The Wellcome Trust Advanced Courses
, USA	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
	http://www.my.americanheart.org	American Heart Association

Science 2006 Scientific Events Calendar

Date	Event	Location
8 – 12 Oct	17th International Symposium on Human Identification	Nashville, TN, USA
8 – 13 Oct	AIPG Annual Meeting	Lexington, KY, USA
10 – 14 Oct	ASHG Annual Meeting	New Orleans, LA, USA
11 – 14 Oct	NABT Annual Meeting	Albuquerque, NM, USA
11 – 15 Oct	Germ Cells	Cold Spring Harbor, NY
13 Oct	ISAP 15th Annual Meeting	Chicago, IL, USA
13 – 17 Oct	Frontiers of Molecular Biology—EMBO Members Workshop	Sheffield, UK
14 – 18 Oct	Neuroscience Annual Meeting	Atlanta, GA, USA
15 – 20 Oct	NOX Family NADPH Oxidases	Les Diablerets, Switzerl
22 – 27 Oct	Biointerface Science	Les Diablerets, Switzerl
22 – 25 Oct	Geological Society of America Annual Meeting and Exposition	Philadelphia, PA, USA
26 – 29 Oct	2006 SACNAS National Conference	Tampa, FL, USA
29 Oct – 2 Nov	AAPS Annual Meeting	San Antonio, TX, USA
30 Oct – 4 Nov	Annual Meeting, AEEG	Boston, MA, USA

November

2 – 5 Nov	Nuclear Receptors and Disease	Cold Spring Harbor, NY
5 – 8 Nov	ACT 27th Annual Meeting	Palm Springs, CA, USA
6 – 9 Nov	BioProcess International™ Conference and Exhibition	San Francisco, CA, USA
12 – 15 Nov	AHS Scientific Sessions 2006	Chicago, IL, USA
15 – 18 Nov	Pharmacogenomics	Cold Spring Harbor, NY
27 Nov – 1 Dec	2006 MRS Fall Meeting and Exhibit	Boston, MA, USA
30 Nov – 3 Dec	Neruodegenerative Diseases: Biology and Therapeutics	Cold Spring Harbor, NY

December

6 – 7 Dec	Biomarkers for Drug Discovery and Development	Amsterdam, Netherlan
9 – 13 Dec	46th American Society for Cell Biology Annnual Meeting	San Diego, CA, USA
9 – 13 Dec	17th International Congress on Parkinson's Disease	Amsterdam, Netherland
11 – 15 Dec	2006 AGU Fall Meeting	San Francisco, CA, US

Visit www.sciencemeetings.org

	For Information	Event Host
.....	http://www.promega.com/geneticidentity	Promega
.....	http://www.aipg.org	American Institute of Professional Geologists, Kentucky Section
.....	http://www.ashg.org	American Society of Human Genetics
.....	http://www.nabt.org	National Association of Biology Teachers
, USA.....	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
.....	http://www.isaponline.org	International Society for Anaesthetic Pharmacology
.....	http:/www.embo.org	European Molecular Biology Organisation
.....	http://www.sfn.org	Society for Neuroscience
land.....	http://www.grc.org/programs/2006/nox.htm	Gordon Research Conferences
land.....	http://www.grc.org/programs/2006/bioint.htm	Gordon Research Conferences
.....	http://www.geosociety.org/meetings/2006/index.htm	Geological Society of America
.....	http://www2.sacnas.org/confNew/confClient/current/	Society for the Advancement of Chicanos and Native Americans in Science
.....	http://www.aapspharmaceutica.com	American Association of Pharmaceutical Scientists
.....	http://www.aegweb.org	Association of Environmental and Engineering Geologists
, USA.....	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
.....	http://www.actox.org	American College of Toxicology
.....	http://www.IBCLifeSciences.com/BPI	IBC Life Sciences
.....	http://www.my.americanheart.org	American Heart Association
, USA.....	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
.....	http://www.mrs.org	Materials Research Society
, USA.....	http://meetings.cshl.edu	Cold Spring Harbor Laboratory
ds	http://www.ibc-lifesci.com/biomarkers	IBC Life Sciences
.....	http://www.ascb.org	American Society for Cell Biology
ds	http://www.parkinson2007.de	World Federation of Neurology
A	http://www.agu.org/meetings	American Geophysical Union

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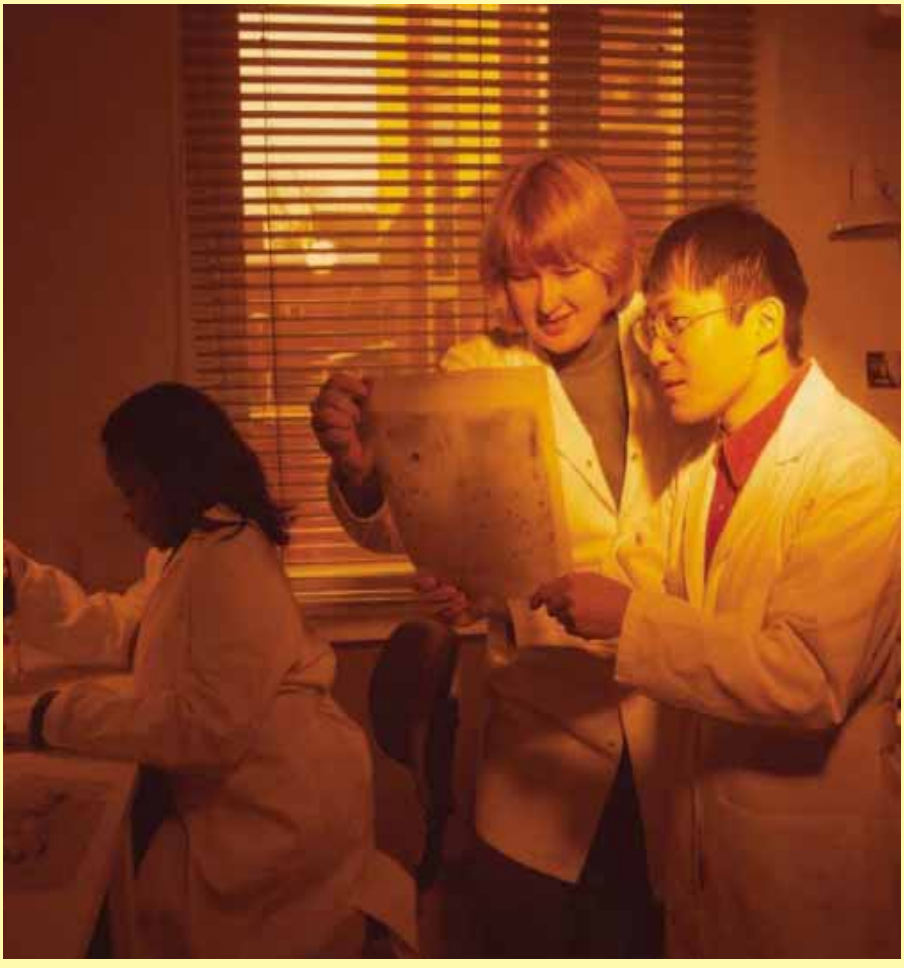
2006 SCIENTIFIC EVENTS CALENDAR



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The Ellison Medical Foundation

Senior Scholar Award in Aging

Request for Letters of Intent – Deadline: March 9, 2006

The Ellison Medical Foundation, established by Lawrence J. Ellison, supports biomedical research on aging. The Ellison Medical Foundation Senior Scholar Program in Aging is designed to support established investigators, working at institutions in the U.S., to conduct research in the basic biological sciences relevant to understanding aging processes and age-related diseases and disabilities. The award is intended to provide significant support to established investigators in order to allow the development of new, creative research programs by investigators who may not currently be conducting aging research or who may wish to develop new research programs in aging. The Foundation particularly wishes to stimulate new research, which has rigorous scientific foundations, but which may not be currently funded adequately, because of its perceived novelty, its high risk, or because it is from an area where traditional research interests absorb most funding. Letters of Intent must be submitted electronically through a link on the Foundation's website on the Applications and Receipt Dates page. The deadline for receipt of the electronic Letters of Intent is **March 9, 2006**.

Areas of interest include, but are not limited to: structural biology, molecular genetics, studies with model systems ranging from lower eukaryotes to humans, inquiries testing the relevance of simpler models to human aging, genetic epidemiology of aging; candidate longevity genes, aging in the immune system, host defense molecules in aging systems, mechanisms of free radical induced cell aging, mechanisms of aging in various differentiated cell populations, gene/environment and gene/gene interactions, integrative physiology, and new approaches to age-modulated disease mechanisms such as those leading to dementias.

Those submitting successful Letters of Intent will be invited to submit full applications. Evaluation is performed by a two phase process involving the Foundation's Aging Initial Review Group and Scientific Advisory Board. Reviewers will pay close attention to arguments as to why the proposed work is unlikely to be supported by established sources. Up to ten Senior Scholar Awards will be made in the Fall, 2006.

Eligibility: Established investigators employed by U.S. 501(c)(3) institutions, or U.S. colleges or universities, are eligible to apply. There is no limit on the number of Senior Scholar letters of intent submitted from any one institution. Current or past Senior Scholar Awardees are not eligible to apply.

Terms of the Award: Each award will be made for up to $150,000 per year direct cost, with full indirect cost at the institution's NIH negotiated rate added to that, for up to four years.

Complete Application Details: Letters of Intent must be submitted electronically through the Foundation's website at **http://www.ellisonfoundation.org**. Instructions and a link to the online Letter of Intent can be found on the Applications and Receipt Dates page.

Address any questions to:

Richard L. Sprott, Ph.D.
Executive Director, The Ellison Medical Foundation
4710 Bethesda Avenue, Suite 204
Bethesda, MD 20814-5226

Phone: 301/657-1830 Fax: 301/657-1828
Email: rsprott@ellisonfoundation.org

Cold Spring Harbor Laboratory
2006 Meetings & Courses

Meetings

Neuronal Circuits: From Structure To Function
March 9 - 12 abstracts due: January 13
Edward Callaway, Dmitri Chklovskii, Liqun Luo

PTEN Pathways
March 15 - 19 abstracts due: January 22
Carlos Cordon-Cardo, Pier-Paolo Pandolfi, Ramon Parsons, William Sellers

Systems Biology: Global Regulation of Gene Expression
March 23 - 26 abstracts due: January 25
Peggy Farnham, Nir Friedman, Jack Keene

Channels, Receptors & Synapses
April 18 - 22 abstracts due: January 25
Richard Huganir, Lily Jan, Morgan Sheng

Gene Expression and Signalling in the Immune System
April 26 - 30 abstracts due: February 1
Doreen Cantrell, Richard Flavell, Rudolf Grosschedl, Stephen Smale

Molecular Chaperones & the Heat Shock Response
May 3 - 7 abstracts due: February 8
James Bardwell, David Ron, Jonathan Weissman

The Biology of Genomes
May 10 - 14 abstracts due: February 15
Kelly Frazer, Thomas Hudson, Svante Paabo, Richard Wilson

The Cell Cycle
May 17 - 21 abstracts due: February 22
Orna Cohen-Fix, Nicholas Dyson, David Morgan

Retroviruses
May 23 - 28 abstracts due: March 1
Frederic Bushman, Jaquelin Dudley

71st Symposium: Regulatory RNAs
May 31 - June 5 abstracts due: March 8
Bruce Stillman, David Stewart

Glia in Health & Disease
July 20 - July 24 abstracts due: April 26
Ben Barres, Martin Raff

Mechanisms & Models of Cancer
August 16 - 20 abstracts due: May 24
Jacqueline Lees, Scott Lowe, Charles Sawyers, Charles Sherr

Molecular Genetics of Bacteria & Phages
August 22 - 27 abstracts due: May 31
Gary Dunny, Tina Henkin, Charles Turnbough, Jr.

Mouse Molecular Genetics
August 30 - September 3
abstracts due: June 5
Francois Guillemot, Janet Rossant, Hiroyuki Sasaki, Anthony Wynshaw-Boris

Translational Control
September 6 - 10 abstracts due: June 5
Alan Hinnebusch, Nahum Sonenberg, Gerhard Wagner

Axon Guidance, Synaptogenesis & Neural Plasticity
September 13 - 17 abstracts due: June 21
Anirvan Ghosh, Christine Holt, Mu-Ming Poo

Dynamic Organization of Nuclear Function
September 27 - October 1 abstracts due: July 5
Genevieve Almouzni, David Spector, Susan Wente

Molecular Genetics of Aging
October 4 - 8 abstracts due: July 22
Judith Campisi, Leonard Guarente, Gary Ruvkun

Germ Cells
October 11 - 15 abstracts due: July 29
Susan Strome, Azim Surani

Nuclear Receptors & Disease
November 2 - 5 abstracts due: August 18
Ronald Evans, Sohaib Khan, Keith Yamamoto

Pharmacogenomics
November 15 - 18 abstracts due: September 1
J. Steven Leeder, Debbie Nickerson, Munir Pirmohamed, Richard Weinshilboum, C. Roland Wolf

Neurodegenerative Diseases: Biology & Therapeutics
November 30 - December 3
abstracts due: September 15
Sam Gandy, Virginia Lee, Marcy MacDonald, Peter Snyder

Spring Courses

applications due January 15

Protein Purification & Characterization
March 29 - April 11
Karen Adelman, Richard Burgess, Albert Courey, Sue-Hwa Lin

Cell & Developmental Biology of *Xenopus*
April 1 - 11
Janet Heasman, Christopher Wylie

Summer Courses

applications due: March 15

Genetics of Complex Human Diseases
June 7 - 13
Ammar Al-Chalabi, Laura Almasy

Advanced Bacterial Genetics
June 7 - 27
John Kirby, Susan Lovett, Anca Segall

Ion Channel Physiology
June 7 - 27
Mark Farrant, Michael Hausser, Nelson Spruston

Molecular Embryology of the Mouse
June 7 - 27
Blanche Capel, Michael Shen

Integrated Data Analysis for High Throughput Biology
June 14 - 27
Harmen Bussemaker, Javier Cabrera, Vincent Carey, Partha Mitra, Mark Reimers, Anirvan Sengupta

Computational Neuroscience: Vision
June 16 - 29
Jonathan Demb, Eero Simoncelli, Stefan Treue

Proteomics
June 30 - July 13
Philip Andrews, Joshua La Baer, Andrew Link

Advanced Techniques in Plant Science
June 30 - July 20
Judith Bender, Lawrence Hobbie, Hong Ma, Sheila McCormick

Neurobiology of *Drosophila*
June 30 - July 20
Greg Bashaw, Scott Waddell, Bing Zhang

Mechanisms of Neural Differentiation & Brain Tumors
July 6 - 12
Abhijit Guha, Sadhan Majumder

Advanced Techniques in Molecular Neuroscience
July 6 - 20
James Eberwine, Thomas Hughes, Cary Lai

Biology of Social Cognition (workshop)
July 14 - 20
Ralph Adolphs, David Skuse

Schizophrenia & Related Disorders (workshop)
July 22 - August 1
Pat Levitt, David Lewis, David Porteous

Eukaryotic Gene Expression
July 25 - August 14
Michael Bulger, Thomas Oelgeschlager, Ali Shilatifard, Laszlo Tora

Imaging Structure & Function in the Nervous System
July 25 - August 14
Florian Engert, Mark Hubener, David Kleinfeld, Jack Waters

Yeast Genetics & Genomics
July 25 - August 14
Frank Luca, Jeffrey Strathern, Malcolm Whiteway

C elegans
July 27 - August 14
Mario de Bono, Arshad Desai, Michel Labouesse

Stem Cells
August 3 - 16
Ronald McKay

Fall Courses

Applications due: July 15

X-Ray Methods in Structural Biology
October 16 - 31
William Furey, Gary Gilliland, Alexander McPherson, James Pflugrath

Programming for Biology
October 18 - 31
Suzanna Lewis, Simon Prochnik, Lincoln Stein, James Tisdall

Immunocytochemistry, In Situ Hybridization & Live Cell Imaging
October 23 - November 5
Abby Dernburg, John Murray, Jason Swedlow

Phage Display of Proteins & Peptides
November 7 - 20
Carlos Barbas, Don Siegel, Gregg Silverman

Computational & Comparative Genomics
November 8 - 14
William Pearson, Randall Smith

For further information:
http://meetings.cshl.edu

Medicines for Malaria Venture
A nonprofit Foundation

FUNDING OPPORTUNITIES

Medicines for Malaria Venture (MMV)

5th CALL FOR LETTERS OF INTEREST

1. **Malaria Drug Discovery Research Proposals**
2. **Malaria Drug Natural Products Proposals**

MMV is a not-for-profit Organization committed to the discovery, development and delivery of affordable antimalarial drugs through public-private partnership. It is expanding its R&D portfolio in order to maximize its chances of delivering a consistent stream of novel antimalarial drugs over the next decade. Two project categories are defined for this year's proposals.

1. **Malaria drug discovery projects** that are directed toward the identification of a candidate compound(s) for entry into preclinical development
   - Projects may be at an exploratory stage but must have an identified target.
   - Projects at an early or late stage of the discovery process are also encouraged to apply.
   - Applications from single institutions (academic or biotech or pharma) or a partnership between an academic centre and a pharmaceutical company are welcome. The key determinant for funding will be the perceived chance of project success.

2. **Malaria Natural Product projects** are also solicited. They are more likely to succeed if applicants have a well-established infrastructure for a natural product programme, and can demonstrate one or more of the following characteristics:
   - Activity of the extracts together with identified compounds, against *P. falciparum in vitro* and in animal models
   - Demonstrated clinical efficacy of originating extract or identified compounds against malaria
   - Methodology for the identification and optimisation of active agent(s).
   - Projects that have already identified active agent(s) are strongly encouraged to apply.

Application for funding is initially requested through submission of a 3 page letter of interest, to reach MMV (see below) no later than **February 28th, 2006**. Electronic submissions are preferred.

Details of this call for the two project categories, as well as project selection process can be obtained from the MMV web site (www.mmv.org) or by directing inquiries to the MMV offices, to the attention of:

Dr. Ian Bathurst
MMV, Rte de Pré-Bois, 20
P.O.Box 1826
CH-1215 Geneva 15
Switzerland
E-mail: ***applications@mmv.org***

MMV gratefully acknowledges the funding and support it has received from: Bill & Melinda Gates Foundation, BHP Billiton, ExxonMobil Foundation, Global Forum for Health Research, International Federation of Pharmaceutical Manufacturers Associations, The Netherlands Ministry for Developmental Cooperation, Rockefeller Foundation, Roll Back Malaria Partnership, Swiss Agency for Development and Cooperation, United Kingdom Department for International Development, United States Agency for International Development, World Bank, World Health Organization, WHO/TDR and Wellcome Trust.

www.mmv.org

CONFERENCE

Human Genetics Society of Australasia

International Federation of Human Genetics Societies

11th International Congress of Human Genetics

August 6-10, 2006
Brisbane Australia

Register now @ www.ichg2006.com

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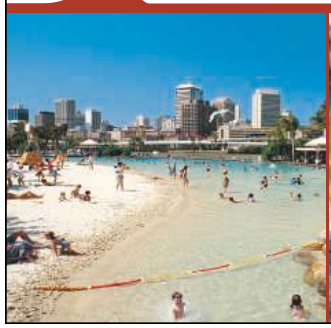

COURSES & CONFERENCES
HINXTON CAMBRIDGE UK

wellcome trust




The Wellcome Trust Conference Programme and Advanced Courses are hosted at the Wellcome Trust Genome Campus – home to one of the world's largest concentrations of expertise in genomics and bioinformatics, including the Wellcome Trust Sanger Institute. The 2006 programme is now open.

COURSES

SPRING 2006

High-Resolution Molecular Cytogenetics
26 February–3 March 2006

Microarrays and the Transcriptome
3–11 April 2006

Open Door Workshop: Working with the Human Genome Sequence
8–10 May 2006

Molecular Basis of Infection: Basic and Applied Research Approaches *NEW
14–20 May 2006

SUMMER 2006

Functional Genomics
21–30 June 2006

Drosophilia Genetics and Genomics
21 June–5 July 2006

Human Genome Analysis: Genetic Analysis of Multifactorial Diseases
19–25 July 2006

Molecular Neurology and Neuropathology (Alternates with Cold Spring Harbor)
20–26 July 2006

AUTUMN 2006

Design and Analysis of Genetic-based Association Studies *NEW
3–7 October 2006

Open Door Workshop: Working with the Human Genome Sequence
13–15 November 2006

OVERSEAS COURSES 2006

Accessing the Human Genome Sequence (Joint with Cold Spring Harbor)
7–9 February 2006, São Paulo, Brazil

Accessing the Human Genome Sequence (Joint with Cold Spring Harbor)
14–16 February 2006, Mexico City, Mexico

Working with Pathogen Genomes
11–14 June 2006, Montevideo, Uruguay

CONFERENCES

SPRING 2006

European Fission Yeast Meeting
16–18 March 2006

SUMMER 2006

Interactome Networks*NEW (Joint meeting with Cold Spring Harbor)
30 August–3 September 2006

AUTUMN 2006

Genomic Perspectives on Host–Pathogen Interactions *NEW (Joint meeting with Cold Spring Harbor)
6–10 September 2006

Genome Informatics *NEW (Joint meeting with Cold Spring Harbor)
Date to be announced

Integrated Biology of Crop Plants *NEW (Joint meeting with European Science Foundation)
8–12November 2006

Vaccine Adjuvants *NEW
Date to be announced

For further information, details of new Courses or Conferences and application details:

www.wellcome.ac.uk/advancedcourses
www.wellcome.ac.uk/conferences

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AWARDS

2006 Weizmann Women & Science Award

Call for Nominations

The American Committee for the Weizmann Institute of Science invites nominations for its 2006 Women & Science Award. The award will be given to an outstanding woman in the U.S. who has made a major contribution to science, technology or engineering. The objectives of the award, which includes a $25,000 research grant, are to recognize distinguished achievement and provide role models to motivate and encourage the next generation of young women in science.

To request a nomination application, please contact Merritt Birnbaum, National Programs, American Committee for the Weizmann Institute of Science, at **212.895.7908**, or by email: **Merritt@acwis.org**. The application must include background documentation, as well as a supporting statement by the nominator. Nominations must be postmarked by January 27, 2006 and sent to: **Women & Science Award, ACWIS, 633 Third Avenue, New York, NY 10017,** or by **fax to 212.895.7993**.

The award ceremony is scheduled to take place in New York City on Monday, June 12, 2006.

Past awardees are:

1994 Joan Argetsinger Steitz, Ph.D.
Henry Ford Professor of Biophysics, Yale University and Investigator, Howard Hughes Medical Institute

1996 Vera Rubin, Ph.D.
Observational Astronomer, Department of Terrestrial Magnetism, Carnegie Institution

1998 Jacqueline K. Barton, Ph.D.
Arthur and Marian Hanisch Memorial Professor of Chemistry, California Institute of Technology

2000 Carla J. Shatz, Ph.D.
Nathan Marsh Pusey Professor and Chair, Department of Neurobiology, Harvard Medical School

2000 Mildred D. Dresselhaus, Ph.D.
Institute Professor of Electrical Engineering and Physics, Massachusetts Institute of Technology (received the Millennial Lifetime Achievement Award)

2002 Susan Solomon, Ph.D.
Senior Scientist, Aeronomy Laboratory, National Oceanic and Atmospheric Administration

2004 May Berenbaum, Ph.D.
Swanlund Professor and Head, Department of Entomology, University of Illinois at Urbana-Champaign

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www.weizmann-usa.org

AWARDS

ROBERT J. AND CLAIRE PASAROW FOUNDATION

2005 MEDICAL RESEARCH AWARDS
Cancer, Cardiovascular Disease, Neuropsychiatry

The Foundation has established three yearly medical prizes for distinguished accomplishment in research in order to increase public awareness of vital areas of investigation. This is the nineteenth year of the awards program. Each award is for $50,000, presented directly to the awardee. The three prizes – one in each of the three fields – are given for basic and/or clinical research.

Cancer: including basic cellular processes and the various forms of cancer. **Past awardees:** Peter K. Vogt, PhD, Irving L. Weissman, MD, George F. Vande Woude, PhD, Erkki Ruoslahti, MD, Harold N. Weintraub, MD, PhD, Ronald M. Evans, PhD, Stanley J. Korsmeyer, MD, Carlo M. Croce, MD, Alfred G. Knudson, Jr., MD, PhD, Robert A. Weinberg, MD, Eric S. Lander, D.Phil., Paul L. Modrich, PhD, Anthony S. Fauci, MD, Alexander J. Varshavsky, PhD, Tom Maniatis, PhD, Roger D. Kornberg, PhD, Elizabeth H. Blackburn, PhD, and Fred W. Alt, PhD.

Cardiovascular Disease: including disorders of the heart and vascular system. **Past awardees:** Burton E. Sobel, MD, Harvey Feigenbaum, MD, Bernardo Nadal-Ginard, MD, PhD, Mordecai P. Blaustein, MD, Jonathan Seidman, PhD and Christine Seidman, MD, Glenn A. Langer, MD, Philip Majerus, MD, Jan L. Breslow, MD, Kenneth R. Chien, MD, PhD, Michael A. Gimbrone Jr., MD, Masashi Yanagisawa, MD, PhD, Mark T. Keating, MD, Eric N. Olson, PhD, Richard P. Lifton, MD, PhD, Robert J. Lefkowitz, MD, Shaun Coughlin, MD, PhD, Judah Folkman, MD, and Barry S. Coller MD.

Neuropsychiatry: including neuroscience of neurologic and mental disorders. **Past awardees:** Nancy Wexler, PhD, Eric R. Kandel, MD, Floyd E. Bloom, MD, Solomon H. Snyder, MD, Michael E. Phelps, PhD, Patricia S. Goldman-Rakic, PhD, Huda Akil, PhD and Stanley Watson, MD, PhD, Arvid Carlsson, MD, PhD, Stanley B. Prusiner, MD, Joseph T. Coyle, MD, Eric J. Nestler, MD, PhD, Fred H. Gage, PhD, Michael I. Posner, PhD and Marcus E. Raichle, MD, Pasko Rakic, MD, PhD, Seymour Benzer, PhD, Tomas Hökfelt, MD, PhD, Thomas M. Jessell, PhD and Judith L. Rapoport, PhD.

The criterion for the Pasarow Medical Research Awards is evidence of extraordinary accomplishment and the likelihood of continuing outstanding achievement in biomedical science.

Nominators for the 2005 Award should provide three sets of materials, each consisting only of a letter of no more than one page stating the rationale for the nomination and a copy of the nominee's curriculum vitae and bibliography. Applications will be reviewed by the Board of Directors in consultation with various medical scholars. Members of the Board of Directors are Jack D. Barchas, MD, President and Chairman; Claire Pasarow, Chief Financial Officer; Brian E. Henderson, MD – University of Southern California; Anthony H. Pasarow – San Pedro, California; Susan Pasarow, MSW – Lake Oswego; Judith L. Swain, MD – UCSD; Joseph P. Van Der Meulen, MD – University of Southern California, and Alexander J. Varshavsky, PhD – CalTech.

Nominations should be sent to: **Robert J. and Claire Pasarow Foundation, c/o Jack D. Barchas, MD, Weill Medical College, Cornell University, 1300 York Avenue, Box 171, Room F–1231, New York, NY 10021**.

Inquiries can be addressed to **Jack D. Barchas, MD** at **(212) 746–3770** or **jbarchas@med.cornell.edu**. Nominations should be received by **January 16, 2006**.

CONFERENCE

Conference on Neural Control of Behavior: Convergent Principles from Divergent Model Systems

February 9-11, 2006, University of California, Los Angeles, CA

The goal of the meeting is to bring together researchers who are asking similar questions, but are using different model systems and different experimental approaches to understand neural control of behavior. This will be an opportunity to get to interact with researchers who share common scientific interests, but have very different perspectives.

Four symposium sessions will focus on the topics of:

- *Neurobiological Basis of Social Relationships:* **Cori Bargmann, Johan Bolhuis, Susan Bookheimer, Hans Hofmann, Lawrence Katz**
- *Fear and Anxiety:* **Robert Adamec, Michael Fanselow, Beat Lutz, Pertti Panula, Lisa Shin**
- *Sleeping and Dreaming:* **Marcos Frank, J. Allan Hobson, Teresa Nick, Jerome Siegel, Giulio Tononi**
- *The Aging Brain:* **Carol Barnes, Caleb Finch, Gary Small, Marc Tatar, Cathy Wolkow**

Keynote speaker: **Thomas Insel**

Organizing committee: Nancy Wayne (chair), **David Glanzman**, and **Kelsey Martin**. Registration deadline is **December 16, 2005**. Travel awards are available for students and postdoctoral fellows.

For more information and registration form, visit our website at:
http://www.physiology.ucla.edu./ncbseminar
Email: **ncb@mednet.ucla.edu**

MBL

Biological Discovery in Woods Hole

Founded in 1888 as the Marine Biological Laboratory

2006 Summer Research Fellowships

Funding Available for Summer Research in Neuroscience

APPLICATION DEADLINE: JANUARY 15, 2006

The MBL is pleased to announce the availability of funding for the following summer research programs in Neuroscience in 2006. These programs will provide up to $50,000/year/award with a possibility for renewal for 3 years. As participants in the MBL's new Neuroscience Institute, scholars in these programs will benefit from the rich intellectual and interactive environment of the scientific community at the MBL.

ALBERT AND ELLEN GRASS FACULTY GRANTS PROGRAM

Proposals must describe collaborative research in any area of neuroscience by teams of two or more investigators, with a minimum stay of six weeks at the MBL in Woods Hole. Collaborative teams must consist of a minimum of two Assistant/Associate Professor-level neuroscientists. Collaborative units may be formed with senior investigators, but only the more junior neuroscientists will receive direct funding. Awards provide funds for research and laboratory rental, and cover the costs of housing and travel for the PIs.

DART FOUNDATION SCHOLARS PROGRAM IN LEARNING & MEMORY

Proposals must be targeted to the study of learning and memory with a minimum stay of six weeks at the MBL. Applications are encouraged from junior- or senior-level neuroscientists holding a Ph.D., M.D. or equivalent degree. Awards provide funds for research and laboratory rental, and cover the costs of housing and travel.

Funding Available for Summer Research

APPLICATION DEADLINE: JANUARY 15, 2006

The MBL is pleased to announce the availability of funding for the following summer research programs in 2006 for junior or senior investigators holding a Ph.D., M.D., or equivalent degree. These prestigious awards provide funds for research and housing. Proposals for Fellowship support will be considered in, but are not limited to, the following fields of investigation:

Cellular & Molecular Physiology
Molecular Biology
Developmental Biology
Neurobiology
Ecology
Parasitology
Microbiology

For application forms and information, contact:
Fellowships Coordinator: fellows@mbl.edu
or call Lenny Dawidowicz, 508.289.7268
MBL, 7 MBL Street, Woods Hole, MA 02543

Applications are encouraged from women and members of underrepresented minorities.
The MBL is an Equal Opportunity/Affirmative Action Employer.

www.MBL.edu/fellowships

CAREERS IN CELL BIOLOGY

Maine Medical Center Research Institute
www.mmcri.org

Faculty Positions

Stem and Progenitor Cell Biology (H601BL) - The Maine Medical Center Research Institute (MMCRI) is expanding its complement of basic and translational research scientists. This campaign is underwritten by two NIH-sponsored COBRE program awards, and by new major commitments by the Maine Medical Center to its research enterprise. Presently available positions are entry-level faculty posts in stem and progenitor cell biology. Primary consideration will be given to candidates with active interests and demonstrated expertise in the areas of self-renewal, fate determination, neoplasia, tissue/organ repair, and lineage commitment. Resources include nationally competitive start-up funds, and core facilities for stem/progenitor cell isolation, DNA & RNA analysis, recombinant viral work, cell imaging, structural biology, and mouse genetics.

Vascular Biology (H605BL) - As part of its continuing expansion, the Maine Medical Center Research Institute (MMCRI) invites applications for one or more positions in the Center for Molecular Medicine at the Scientist I, Scientist II and Senior Scientist (assistant, associate and full professor equivalent) levels. Center research focuses on clinically relevant problems in cell signaling, protein trafficking, morphogenesis/differentiation, stem cell and cancer biology in the contexts of hematopoietic and vascular development and homeostasis. These recruitments are facilitated by two NIH-sponsored COBRE awards and by new major commitments by the Maine Medical Center to expand basic, translational, and clinical research. Scientists using cutting-edge molecular, cellular, biochemical and genetic approaches in these areas are strongly encouraged to apply. Candidates must have a Ph.D. and/or M.D. degree. Candidates at the Scientist I level must have three or more years of postdoctoral experience, an outstanding publication record, and strong potential for extramural funding. Candidates at the Scientist II and Senior Scientist levels must have an outstanding national record of academic achievement and an established, independent, extramurally-funded research program. Successful candidates will receive nationally competitive start-up packages and access to excellent core facilities for DNA and protein analysis, flow cytometry/cell sorting, confocal microscopy, histopathology, mouse transgenics and magnetic resonance imaging. Appointees will be encouraged to interact with basic, translational, and clinical research programs at the Maine Medical Center and to participate in graduate education programs.

Benefits, salary and resources are nationally competitive and positions are within the Maine Medical Center Research Institute, **www.mmcri.org**. Productive investigators with relevant experience should submit applications to: **Maine Medical Center, Employment Office, 22 Bramhall St., Portland, ME 04102; Fax: (207) 662-4999. Candidates can also apply online at: www.mmcri.org or http://jobs.mmc.org**

The MaineHealth Family, EOE

CONFERENCE

3RD INTERNATIONAL CONFERENCE ON STRUCTURAL ANALYSIS OF SUPRAMOLECULAR ASSEMBLIES BY HYBRID METHODS

March 15-19, 2006 • Lake Tahoe, CA, USA
(www.burnham.org/hybridmethods2006)

PROGRAM SESSIONS

- Computational Approaches to Hybrid Analysis
- Hybrid Approaches to Dynamic Assemblies
- Hybrid Approaches to Macromolecular Machines
- Cellular Proteomics
- Hybrid Approaches to Macromolecular Filaments
- Hybrid Approaches to Membarnd Complexes
- Hybrid Approaches to Dynamic Macromolecular Assemblies and Machines

CONFERENCE DEADLINES

Dec 15, 2005: Late Breaking Abstract Deadline
Feb 15, 2006: Conference Registration Deadline

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SYMPOSIA

UT SOUTHWESTERN MEDICAL CENTER

The Department of Biochemistry
The University of Texas Southwestern Medical Center
Announces:
"Celebration of Biochemistry"
a Symposium honoring the scientific careers of
Ronald W. Estabrook, Ph.D. and John M. Johnston, Ph.D.
Friday, April 21, 2006

Excellence in Education Foundation Auditorium, NB2.102
The University of Texas Southwestern Medical Center
6000 Harry Hines Boulevard, Dallas, TX 75390

Symposium Speakers:

Dr. David Baker, University of Washington
Dr. Arthur E. Johnson, Texas A&M University
Dr. Gregory Petsko, Brandeis University
Dr. Erick M. Carreira, Laboratorium fuer Organische Chemie, Zurich, Switzerland
Dr. Andrew G. Myers, Harvard University
Dr. Harry B. Gray, California Institute of Technology
Dr. Joan A. Steitz, Yale University
Dr. Joanne Chory, Salk Institute for Biological Studies
Dr. Phillip A. Sharp, Massachusetts Institute of Technology

For additional information and registration, please see our website:
http://biochemistry.swmed.edu/Symposium/index.php

Or contact:
Casandra Moxey, Department of Biochemistry
(214) 648-4744, Casandra.Moxey@UTSouthwestern.edu

CAREERS IN CELL BIOLOGY

COLUMBIA UNIVERSITY
TENURE-TRACK FACULTY POSITIONS

The Department of Physiology and Cellular Biophysics at Columbia University invites applications for three tenure-track faculty positions at the Assistant, Associate, and Full Professor levels. The department plans to expand its expertise in vertebrate and nonvertebrate systems. Our ideal candidate is someone who uses cellular-based studies in attempting to analyze larger questions of systems biology; for example, studies of cardiovascular biology, muscle molecular mechanics, differentiation, signal transduction, and organogenesis. Studies aimed at identification of molecular and cellular basis of disease are especially welcome. Because the department is already strong in neurobiology, applicants from different disciplines will be given preference. Physiology Professor James Rothman is leading the Columbia Genome Center and launching a new initiative in high throughput cell-based screening of genetic pathways of cell function and disease pathogenesis that will provide a rich environment facilitating rapid discovery of critical pathways. In particular, the Columbia Genome Network has been selected to be a part of an NIH roadmap consortium, the Molecular Library Screening Network, which shares this vision (http://www.genome.gov/15014443).

M.D., Ph.D., or equivalent degree required. Visit the departmental Web site at http://www.healthsciences.columbia.edu/dept/physio/physio2/

All correspondence must be sent electronically. Please send CV, three references, cover letter, and a brief statement of current and future research interests to:

Qais Al-Awqati M.D.
Chair Physiology Search Committee
e-mail: ma2199@columbia.edu

Columbia University is an affirmative action/equal opportunity employer.

GRADUATE PROGRAM

Drew University
Residential School on Medicinal Chemistry: Chemistry and Biology in Drug Discovery
Madison, New Jersey – June 12-16, 2006

The Residential School is a week-long graduate level course organized to provide an accelerated program for medicinal chemists and biologists who wish to broaden their knowledge of small molecule drug discovery and preclinical development. The course is focused on fundamental concepts that are useful in drug discovery spanning initial target validation, enzyme and receptor assays, high throughput screening, hit-to-lead progression, lead profiling and modification, structure-based drug design, QSAR, plasma protein binding, pharmacokinetics, metabolism, drug delivery, toxicology and patents. Case histories of recent successful drug discovery and development programs will also be presented. Attendance is limited to 200 participants with preference given to applicants having five years or less industrial experience.

A Special Topics Course on **Designing Drugs with Optimal *In Vivo* Activity After Oral Administration** is also being offered on **June 19-20**. This course, which is limited to 40 participants, will cover topics relevant to the design of orally active drugs including GI physiology, oral bioavailability, formulation, prodrug strategies, pharmacokinetics and pharmacodynamics, intestinal and hepatic metabolism, biliary and renal clearance, and appropriate case histories. Previous attendance at the Residential School on Medicinal Chemistry is not a prerequisite for acceptance to the Special Topics Course.

More information and application forms can be obtained at www.depts.drew.edu/resmed or by contacting the Residential School's Office at Drew University, Hall of Sciences, Room 319, Madison, NJ 07949, USA; Phone: 973/408-3787, Fax: 973/408-3504, E-mail: resmed@drew.edu

FACULTY,
Department of Cell Biology

The Department of Cell Biology at Duke University Medical Center invites applications for a tenure-track position at the Assistant Professor Level. We are seeking candidates working with a metazoan system in one of the following two areas: 1) Cell polarity, with an emphasis on mechanistic studies; 2) Biology of the nucleus, with an emphasis on structural/functional organization.

Candidates must have a strong record of achievement and a well-formulated and innovative research plan. The Department is in a major expansion phase and successful applicants will enjoy newly renovated space and a stimulating and supportive research environment.

Applications will be considered as they are received up to January 30th, 2006. Please include CV, brief summary of past and future research, and reprints of recent papers, and arrange for three letters of support to be sent directly to: **Dr. Brigid LM Hogan, Chair, Department of Cell Biology, Room 388C, Nanaline H. Duke Building, Box 3709, Duke University Medical Center, Durham, NC 27710**. Duke University Is An Equal Opportunity/Affirmative Action Employer.

Duke University
Medical Center

ANU
THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH ASSOCIATE

Faculty of Science
School of Biochemistry & Molecular Biology
ARC Centre of Excellence in Plant Energy Biology

Academic Level A or B
Fixed term – 2 to 5 years
Salary Range: $49,690 - $74,313 pa plus 17% super
Reference: FS 3115

The ARC Centre of Excellence in Plant Energy Biology is an ambitious new enterprise incorporating seven teams at The University of Western Australia, The Australian National University and The University of Sydney. The Australian National University incorporates two research teams lead by Murray Badger and Barry Pogson, undertaking research relating to chloroplasts, photosynthetic function and plant organelle metabolism in model plants using functional genomics. We are seeking to appoint up to three Research Associates for up to 5 yrs at the ANU to carry out research in one or more areas of molecular/cell biology, biochemistry or genetics.

There are a number of potential project areas which are addressed in the further particulars and the successful applicant will be expected to develop a strong research program that complements existing research in one or more of these areas, to co-supervise graduate students, and to contribute to the development and coordination of the Centre's research.

Selection Criteria: http://info.anu.edu.au/hr/jobs/ or from Marie McNamara T: +61 2 6125 2284
E: Marie.Mcnamara@anu.edu.au

Enquiries: Murray Badger T: +61 2 6125 3741
E: Murray.Badger@anu.edu.au or Barry Pogson T: +61 2 6125 5629
E: Barry.Pogson@anu.edu.au

Closing Date: Monday 16 January 2006

2004

EOWA EMPLOYER OF CHOICE FOR WOMEN

The University's diverse workforce contributes to its success. Applications from Aboriginal and Torres Strait Islanders, people with disabilities, people from culturally and linguistically diverse backgrounds, and women and men in non-traditional occupations are keenly sought.

CRICOS# 00120C

Information about the Centre can be viewed at http://www.plantenergy.uwa.edu.au

The University of Georgia

New Faculty Positions in Biomedical Sciences

The University of Georgia is dramatically expanding its research programs in interdisciplinary biomedical and health sciences and is recruiting tenure track or tenured faculty in a number of related areas. This exciting initiative is marked by expansion of the Biomedical Health Sciences Institute, the opening of the Paul D. Coverdell Center for Interdisciplinary Biomedical Studies, the establishment of a new College of Public Health, the development of new, state-of-the-art bio-containment facilities for studies of animal and human health, and increasing emphasis on quantitative approaches to biological and medical problems. The University seeks to expand existing strengths by recruiting faculty in the following areas:

- **Genetics of complex traits relevant to human health and disease**
- **Pathogenesis, bioinformatics or host cellular response to emerging and re- emerging bacterial infectious diseases**
- **Immunology**
- **Molecular epidemiology**
- **Public Health: biostatistics, epidemiology, and health policy**
- **Systems biology, especially combined use of experimental and computational approaches to studies of signaling in complex contexts, such as cancer**
- **Glycoscience, especially glycosaminoglycan structure/function, glyco-immunology, cell/developmental, cancer or chemical biology**
- **Drug discovery**

Successful candidates will have the opportunity to participate in a rich interdisciplinary and collegial environment provided by the center and institute structure at UGA (described at **http://www.uga.edu/research/centers1.html**), and to benefit from partnerships involving nearby institutions, including the Centers for Disease Control and Prevention, the Medical College of Georgia, Georgia Tech and Emory University.

Applicants for any of these positions must have an advanced degree (PhD, DVM, MD or equivalent). Successful candidates will be expected to establish nationally recognized, externally funded research programs and contribute to teaching in undergraduate, graduate and professional training programs.

For more information regarding these positions, and for application instructions, please visit: **http://www.ovpr.uga.edu/facultypositions/**.

The University of Georgia is an Affirmative Action and Equal Opportunity Employer.

THE BRAIN INSTITUTE
at the University of Utah

ASSISTANT PROFESSOR CELLULAR NEUROSCIENCE

The Brain Institute at the University of Utah, in collaboration with the Departments of Biology, Neurobiology & Anatomy and Physiology, invites applications for a tenure-track faculty position at the Assistant Professor level. We seek a creative and interactive individual working on fundamental problems in cellular neuroscience. Successful applicants will be expected to establish a vigorous independent research program and contribute to teaching of graduate or undergraduate students in his or her area of expertise. New faculty will have access to multiple graduate admissions programs and outstanding infrastructural support covering a wide variety of scientific disciplines.

Please submit a CV, representative publications, descriptions of research and teaching interests and three letters of reference to: Erik Jorgensen, Chair, Cellular Neuroscience Search Committee, The Brain Institute at the University of Utah, 417 Wakara Way, Suite 2210, Salt Lake City, UT 84108, or braininstitute@utah.edu. The candidate must hold a Ph.D. or M.D. degree. Review of applications will begin February 1, 2006 and will continue until the position is filled.

The University of Utah is an Equal Opportunity/ Affirmative Action employer, encourages applications from women and minorities and provides reasonable accommodation to the known disabilities of applicants and employees.

http://brain.utah.edu

The University of Edinburgh

The University of Edinburgh is an exciting, vibrant, research-led academic community offering opportunities to work with leading international academics whose visions are shaping tomorrow's world.

Research Group Leader

The Wellcome Trust Centre for Cell Biology seeks a Research Group Leader with an interest in fundamental cellular processes.

Based at the King's Buildings campus of Edinburgh University, Centre scientists study cellular function using the techniques of biochemistry, molecular biology, genetics and microscopy. You will have access to state-of-the-art instrumentation in an international and highly collegiate research environment. The Centre benefits from interactions with the flourishing scientific community of Edinburgh University and its associated Research Institutions.

Informal enquiries may be directed to Professor Bird, e-mail: Christine.struthers@ed.ac.uk

Please send a brief research proposal and CV including the names of three academic referees to Professor Adrian Bird (Director), Wellcome Trust Centre for Cell Biology, University of Edinburgh, Michael Swann Building, The Kings Buildings, Edinburgh EH9 3JR.

The deadline for applications is 23 December 2005. Shortlisted candidates will be invited for interview soon after that date.

Committed to Equality of Opportunity

www.jobs.ed.ac.uk

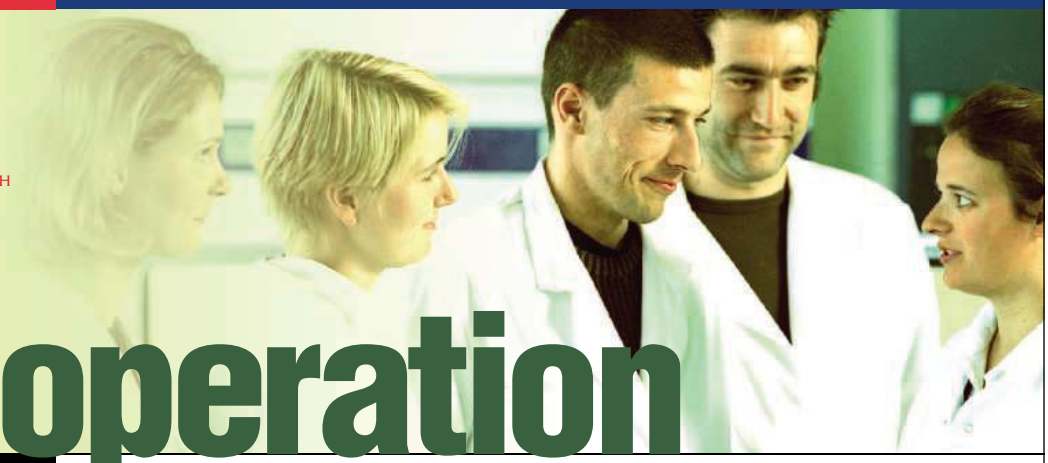
Director - Institute of Molecular Biophysics Florida State University

A well-established biomolecular scientist is sought to lead the Institute of Molecular Biophysics (IMB). The Institute's focus is biomolecular structure/function, probed through crystallography, NMR, EPR, electron microscopy, mass spectrometry and computation. A newly renovated 50,000 ft^2 building houses 12 research groups, 4 core research facilities, and serves as the home for the Molecular Biophysics Doctoral Program and an inter-departmental Structural Biology research program of ~ 30 faculty (**www.sb.fsu.edu**). Faculty of the Institute have academic appointments in the departments of Biological Science, Chemistry and Biochemistry, Physics and Mathematics. We seek to further develop strong ties with other campus initiatives: the National High Field Magnet Laboratory (**www.magnet.fsu.edu**), the School of Computational Science (**www.csit.fsu.edu**), the Center for Materials Research Technology (**www.martech.fsu.edu**), a new Molecular Recognition program (**www.chem.fsu.edu**) and Biomedical Science in the new College of Medicine (**www.med.fsu.edu**).

The Director will lead the Institute in its future growth, its research and graduate education mission and will foster interdisciplinary/inter-departmental initiatives. The candidate's research interests should be in an area of cellular or molecular science where molecular biophysics has growing impact, but need not be the primary focus. Nominations and applications are welcome.

Applications should consist of a curriculum vitae, brief statement of experience and scientific goals and contact information for four potential referees. They should be sent to: **Michael S. Chapman, IMB Director Search Committee, Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306-4380; (chapman@sb.fsu.edu)**.

Florida State University is an Equal Opportunity/Affirmative Action Employer, committed to diversity in hiring, and a Public Records Agency.



cooperation

Johnson & Johnson Pharmaceutical Research & Development (J&JPRD) is a global research and development organization in neuroscience, oncology, infectious diseases, and internal medicine research with research sites both in the United States of America and Europe.

To build further on our success, the J&J PRD site in Beerse, Belgium needs to recruit new scientists who have the creativity to develop the medicines of tomorrow.

Our strategy is to create a pool of postdoctoral positions from which recruitment for new permanent positions may take place in the near future.

For the next generation of scientists and leaders in pharmaceutical research, J&JPRD is an exciting and scientific challenging place to work.

You'll have the support of multidisciplinary culture that believes in making the unattainable a reality every day.

Postdoctoral positions - Scientists
Medicinal Chemistry/Organic Chemistry

(M/F) **Ref. 2005/P051557/Science**

Job Description:

- As part of a medicinal chemistry project team you will hands-on design, validate and synthesize novel biologically active compounds and libraries using state-of-the-art synthesis and combinatorial strategies.
- Your research is expected to contribute to the identification and optimization of novel molecular entities for clinical evaluation in the fields of CNS, internal medicine and oncology.

Your Profile:

- The ideal candidate will have a PhD in organic chemistry or medicinal chemistry with experience in multi-step organic synthesis, purification techniques and structural characterization of organic molecules. Hands-on experience in solution or solid-phase parallel synthesis is a plus.
- The ability to work in a small multidisciplinary research team as well as effective communication skills and a good mastery of the English language is essential.

Additional info:

- The position can start immediately and will terminate after 3 years.
- J&JPRD, Beerse, Belgium is offering a competitive salary package and a scientific challenging environment with state-of-the-art equipment and brand new laboratory facilities.
- J&JPRD is proud to be an equal opportunity employer.

For additional information, please phone Dr. Lieven Meerpoel on +32 (0) 14 60 56 94,
or send an e-mail to: lmeerpoe@prdbe.jnj.com

Does this opening appeal to you?

If so, send us a detailed CV right away, preferably by e-mail, to: Lieven Meerpoel (lmeerpoe@prdbe.jnj.com).
Don't forget to state the reference no. for the position. If you want, you can apply in writing to:
Medicinal Chemistry Department, J&JPRD - a division of Janssen Pharmaceutica NV,
For the attention of Lieven Meerpoel, Turnhoutseweg 30, B-2340 Beerse, Belgium.
A confidential fax can be sent to +32 (0) 14 60 53 44. Our website: http://www.janssenpharmaceutica.be/jobs.

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

Health Research in a Changing World

Fighting Diseases and Improving Lives

Tenure Track Position in Bacterial Pathogenesis
Laboratory of Clinical Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Department of Health & Human Services

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR), Laboratory of Clinical Infectious Diseases (LCID) is seeking an outstanding investigator to develop a clinical and basic program in bacterial pathogenesis.

The LCID studies the pathogenesis, pathophysiology, treatment and prevention of infectious diseases, including emerging infections and pathogens that are of concern in biodefense, as well as microorganisms that cause persistent, recurrent, or fatal disease. Current areas of clinical and basic expertise in the LCID include viral, fungal, and mycobacterial pathogenesis and pathophysiology and the pathophysiology of defects in cellular apoptosis.

The successful candidate will establish an independent research program in bacterial pathogenesis with both laboratory and clinical components. The incumbent will develop clinical protocols, which may include natural history, pathophysiology, mechanism of action, treatment, or all of the above. Board eligibility/board certification or the equivalent in Internal Medicine or Pediatrics and Infectious Diseases or Allergy and Immunology are desirable, but Ph.D.'s with active clinical programs are also encouraged to apply. Sufficient independent resources including space, support personnel and an annual budget for services, supplies and salaries have been committed to the position to ensure success.

The appointment is a Tenure Track appointment and will be at the appropriate level under Title 42, which is equivalent to a University Assistant Professor rank. Salary is dependent on experience and qualifications.

Interested candidates may contact Dr. Steven Holland, Chief, LCID, DIR, and NIAID at 301/402-7684 or email (smh@nih.gov) for additional information about the position.

To apply for the position, candidates must submit a curriculum vitae, bibliography, three letters of reference, a detailed statement of research interests, and reprints of up to three selected publications by January 31, 2006 to Patrick Murray, Ph.D., Chair, NIAID Search Committee, c/o Mrs. Lynn Novelli, Committee Manager, 10 Center Drive, MSC 1356, Building 10, Room 4A26, Bethesda, Maryland 20892-1356. Further information on this position and guidance on submitting your application is available on our website at: http://healthresearch.niaid.nih.gov/science

Please reference "Science" on your resume.

National Institute of General Medical Sciences
National Institutes of Health

The National Institute of General Medical Sciences (NIGMS) in Bethesda, MD is seeking applications from outstanding candidates for a Health Scientist Administrator (HSA) position in the Pharmacological and Physiological Sciences Branch within the Pharmacology, Physiology, and Biological Chemistry Division. The recruiting branch currently supports research and training into understanding the basis of traumatic and burn injury and the perioperative period, the molecular basis of action of anesthetics, the mechanisms of and genetics underlying the actions of therapeutic drugs, and the development of predictive preclinical toxicology approaches.

The individual hired will be responsible for applying his/her clinical and research expertise to manage and develop research and training grants in NIGMS' broad areas of basic studies in pharmacological and physiological sciences, and to foster the translation of results from fundamental research areas into clinical studies. The person should have experience gained in a medical research institution and understand how research is conducted with human subjects or patients in a clinical setting. A background in at least one of the following areas is preferred: trauma, injury and recovery, or clinical pharmacology, or immune system biology, or alternatively in a cross-cutting area such as studies of the role of inflammation in the disease process or of molecular/cellular signaling in these systems. Experience in modern methods of genome or proteome analysis would also be desirable.

Applicants must possess an MD and/or PhD plus scientific knowledge in the fields of pharmacology, physiology, immunology, systems biology, medicine, or related fields. Applicants must be familiar with both clinical and laboratory approaches in his/her own field(s) of expertise. Experience in the NIH peer review and grant award process would be beneficial. Salary will be commensurate with qualifications, may include a physician's comparability allowance, and will have a full package of benefits. Detailed vacancy announcements **NIGMS-05-100271** and **NIGMS-05-100881** with the qualifications and application procedures are available at the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html**. Questions about application procedures may be directed to **Erin Bandak at 301-594-2324**. Applications must be received by **January 4, 2006**.

WWW.NIH.GOV

The National Institute of Allergy and Infectious Diseases (NIAID), a major research component of the NIH and the Department of Health and Human Services, is recruiting for a Post-doctoral Fellow or Research Fellow. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D., Ph.D., or DVM are eligible. The Research activity involves (1) the development of live attenuated parainfluenza virus vaccine candidates and their characterization in rodents, in non human primates, and in humans; (2) the use of new "rescue" systems for these viruses to examine basic questions of viral genetics, molecular virology, viral pathogenesis, and the molecular basis of attenuation; (3) production of new candidate vaccines using site-directed mutagenesis to introduce desired attenuating mutations into viral genomes; and (4) the evaluation of the immunologic determinants of resistance to infection and illness caused by these parainfluenza viruses. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to applied vaccinology, and they provide excellent training for newly graduated Ph.D. scientists, for postdoctoral scientists, and for MD's at all levels of training who plan a career in research in infectious diseases. The salary range for post-doctoral applicants is $38,500-56,900, depending on experience. Research Fellow applicants should have three or more years of post-doctoral experience; the salary range is $40,974-72,990. Applicants with an MD degree are eligible for the NIH Loan Repayment Program. Applicants should send their curriculum vitae, a letter of interest, and names and addresses of three (3) references to **Brian Murphy, 50 South Drive Room 6517 MSC 8007, Bethesda, MD 20892-8007, FAX: (301) 480-1268, email: bm25F@nih.gov.**

The National Institute of Allergy and Infectious Diseases (NIAID), a major research component of the NIH and the Department of Health and Human Services, is recruiting for one Post-doctoral Fellow or Research Fellow. The position will be in the Epidemiology Section of the Laboratory of Infectious Diseases. The research program focuses on epidemiology, molecular biology, host immune response, and vaccine development for the human noroviruses. The salary range of post-doctoral applicants is $38,500-56,900, depending on experience. Research Fellow applicants should have three or more years experience; the salary range is $40,974-72,990. Applicants should send their curriculum vitae and contact information for three (3) references to **Kim Y. Green, 50 South Drive MSC 8026, Room 6318, Bethesda, MD 20892-8007, FAX: (301) 480-5031, email: kgreen@niaid.nih.gov.**

Health Scientist Administrator
National Institute of General Medical Sciences

The National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland is seeking applications from outstanding candidates for one Health Scientist Administrator position in the Division of Pharmacology, Physiology and Biological Chemistry, which supports primarily basic, non-disease-oriented research and training, including a substantial portfolio of bio-related chemical research.

The incumbent for this position will be responsible for developing and managing a portfolio of research grants that support studies that utilize organic chemistry to understand and/or control biological systems. The ideal candidate will have a substantial background in the design, synthesis and evaluation of small organic molecules as well as a thorough grounding in such areas as biochemistry, pharmacology or molecular biology. Prior research experience in bio-related organic chemistry is desirable.

Applicants must possess a Ph.D. or M.D. plus scientific knowledge and demonstrated expertise in at least one of the following areas: organic chemistry, biochemistry, pharmacology, or related areas, and knowledge of the NIH peer review and grants process. Salary is commensurate with qualifications, and includes a full package of benefits. A detailed vacancy announcement **(NIGMS-05-100284)** with the mandatory qualifications and application procedures can be obtained via the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html** and NIH Home page at **http://www.jobs.nih.gov**. Questions on application procedures may be addressed to **Erin Bandak at (301) 594-2324**. Applications must be received by close of business **January 6, 2006**.

NIH Women's Health Clinical Fellowship

Qualified doctoral-level scientists are invited to apply for a newly established clinical fellowship at the National Institutes of Health that will focus on training scientists to address key issues in women's health research. For a list of potential mentors and research projects, see <http://orwh.od.nih.gov/clinfellowships>.

The fellowship offers not only the opportunity to carry out clinical, epidemiological or translational research but also to participate in relevant clinical rotations and in the NIH-Duke Masters Training Program in Clinical Research. Fellows will be expected to attend the Introduction to the Principles and Practice of Clinical Research course during their first year. Available to doctoral-level fellows within five years of receipt of the doctoral degree. Must be U. S. citizen or permanent resident.

Applications must be made on-line, by **January 15**, at:
<http://www.training.nih.gov/transfer/WomensHealthAds>

NIH Partners
Intramural Program on Research On Women's Health
Office of Research on Women's Health
Office of Intramural Research

Private Partner
Foundation for NIH
with a grant from AstraZeneca

GSI Darmstadt

the German National Laboratory for Heavy-Ion Research, member institute of the Helmholtz Association of German Research Centres, invites applications in a joint appointment with the

Ruprecht-Karls-Universität Heidelberg

for a

Leading Scientist and Professor (W3) for Experimental Atomic Physics

(as successor to Jürgen Kluge)

The candidate will represent the field of atomic physics in research and teaching, will lead the Atomic Physics Group at GSI and will further develop the cooperation with national and foreign research institutes. The professorship at the Ruprecht-Karls-Universität in Heidelberg is with the Institute of Physics in the Faculty of Physics and Astronomy.

Presently, the main focus of research of the Atomic Physics Group is the investigation of the atomic structure and of atomic reactions of highly-charged ions as well as the determination of nuclear ground-state properties exploiting atomic-physics methods. For this purpose modern techniques are being developed and implemented for storage, cooling and detection of ions in storage rings and traps, for detection of photons in the optical and hard X-ray spectral region as well as for laser spectroscopy. The future accelerator facility FAIR (**F**acility for **A**ntiproton and **I**on **R**esearch) at GSI will open up new opportunities in carrying out atomic-physics experiments with low-energy antiprotons.

The position is covered by a post for a permanent employment contract. The holder of the position will be granted leave after the appointment of professorship at the University of Heidelberg to pursue the assignment at GSI. In accordance with §67, paragraph 1 UG, all initial appointments of professorships must, as a basic principle, be limited in time. Exceptions are possible, particularly when foreign applicants or applicants from outside the university community cannot otherwise be acquired. Should the employment status be continued after the expiration of the time limit, it will not be necessary to re-apply for the appointment.

The advancement of women in the scientific field is an integral part of the university's and GSI's policy. Women, therefore, are especially encouraged to apply.

Persons with disabilities will be given preference over other applicants with comparable qualifications.

Applications, including CV and publications list, should be submitted by postal mail in two copies by **December 23, 2005** to:

Professor Dr. W. F. Henning
Scientific Director of
Gesellschaft für Schwerionenforschung mbH
Planckstr. 1
64291 Darmstadt
Germany

GRADUATE PROGRAM

Graduate School of GENOME SCIENCE & TECHNOLOGY
The University of Tennessee (UT) & Oak Ridge National Laboratory (ORNL)
http://gst.ornl.gov/

Seeking Outstanding Students

- Full tuition waiver and Health Benefits
- Stipend of $18,000
- Accepting applicants with biological, physical, or computational backgrounds

Interdisciplinary Program

- UT and ORNL facilities/expertise
- Seventy-five faculty
- Academic environment and team approaches

Areas of Study

- Genetics/Genomics
- Structural Biology/Proteomics
- Computational Biology/ Bioinformatics
- Bioanalytical Technologies

To apply visit the website:
http://gst.ornl.gov
Or Write: Dr. Cynthia B. Peterson, Director
UT/ORNL Graduate School of Genome Science & Technology
1060 Commerce Park
Oak Ridge, TN 37830-8026
cbpeters@utk.edu

FELLOWSHIPS FOR POSTDOCTORAL SCHOLARS AT WOODS HOLE OCEANOGRAPHIC INSTITUTION

Fellowships are available to new or recent doctoral graduates in diverse areas of research. Interested persons are encouraged to submit applications no later than January 15, 2006. Applications will be accepted from doctoral recipients with research interests associated with the following:

Departments - Applicants who wish to conduct research on topics of general interest to one or more of the departments are encouraged to apply. Four to five awards are anticipated. The Departments are:

- **Applied Ocean Physics & Engineering**
- **Biology**
- **Marine Chemistry & Geochemistry**
- **Geology & Geophysics**
- **Physical Oceanography**

Institutes - With the aim of fostering interdisciplinary research addressing critical issues, WHOI has established four institutes. We anticipate that we will award a fellowship to support research associated with each of the Institutes. The Institutes are:

- **Coastal Ocean Institute**
- **Deep Ocean Exploration Institute**
- **Ocean and Climate Change Institute**
- **Ocean Life Institute**

The NOAA-WHOI Cooperative Institute for Climate & Ocean Research (CICOR) will award a Fellowship in one of three theme areas: Coastal Processes; Climate; Marine Ecosystems.

Recipients of awards are selected competitively, with primary emphasis placed on research promise. Fellowships are awarded for 18-month appointments with a stipend of $52,000 per year, a modest research budget and eligibility for group health insurance. Recipients are encouraged to pursue their own research interest in association with Resident Scientific and Senior Technical Staff. Communication with potential WHOI advisors prior to sumbitting an application is encouraged. Completed applications must be received by January 15, 2006 for the 2006/2007 appointments. Awards will be announced by March 31, 2006.

Further information about the Fellowships and application forms as well as links to the Individual Departments, Institutes and Centers and their research themes may be obtained through the Academic Programs section of the WHOI web pages at: http://www.whoi.edu/apo, or by writing directly to:

Postdoctoral Fellowship Committee
Academic Programs Office, MS #31
Woods Hole Oceanographic Institution
266 Woods Hole Road
Woods Hole, MA 02543-1541
Telephone: (508) 289-2219
Fax: (508) 457-2188
E-mail: postdoc@whoi.edu
Internet: http://www.whoi.edu/apo

Woods Hole Oceanographic Institution

Equal Opportunity/Affirmative Action Employer

Tenure-Track Faculty Positions
Department of Pharmacological and Pharmaceutical Sciences
College of Pharmacy, University of Houston

The Department of Pharmacological and Pharmaceutical Sciences at University of Houston College of Pharmacy is accepting applications for two tenure-track faculty positions, one at the Assistant or Associate Professor level in integrative cardiovascular/renal pharmacology/physiology and the other at the Assistant, Associate or Full Professor level in drug transport/metabolism and drug delivery. However, applicants with relevant expertise in associated areas are encouraged to apply. The University of Houston is a large and diverse urban research university, and the College of Pharmacy has a presence at both the main campus, and the nearby Texas Medical Center. Department faculty members teach in the Pharm.D. professional program of the College of Pharmacy. In addition, the department has graduate programs in both Pharmacology and Pharmaceutics, leading to the Ph.D. and Pharm.D./Ph.D. degrees.

Eligible candidates must have an earned doctoral degree and postdoctoral experience. They also must have a funded research program or show strong potential for developing one.

Applications will be accepted until the position is filled, although an early application is recommended. Interested individuals should send a letter describing his/her research program, a curriculum vitae, representative publications, and the names of three references with postal and e-mail addresses, telephone and FAX numbers to: **Yuen-Sum (Vincent) Lau, Ph.D., Chair, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, College of Pharmacy, 521 Science Research Bldg 2, Houston, TX, 77204-5037; Web site: http://www.uh.edu/pps/ppsp/index.html.**

The University of Houston is an Affirmative Action/Equal Opportunity Employer. Minorities, women, veterans, and persons with disabilities are encouraged to apply.

University of Houston
Learning. Leading.

10YR ANNIVERSARY

جامعة كارنيجي ميلون قطر
Carnegie Mellon QATAR CAMPUS
مؤسسة قطر
Qatar Foundation

Human-Computer Interaction Visiting Faculty Position in the School of Computer Science

Carnegie Mellon University established a branch campus in Qatar in the fall of 2004. We are offering a BS degree in Computer Science to an international student body. The university invites applications for a visiting faculty position to begin as early as January 2006.

We are seeking a faculty member in the area of Learning Science and Technology with research experience ideally in designing, implementing, deploying, and evaluating educational technology in school or college settings. An ability to teach courses in human-computer interaction, artificial intelligence, cognitive psychology, or related areas is also desired. The position will involve research in collaboration with the Pittsburgh Science of Learning Center and faculty at the Human-Computer Interaction Institute at Carnegie Mellon in Pittsburgh. The position offers competitive salaries, overseas assignments, travel and housing allowances and other benefits packages, as well as attractive research support.

Interested candidates should send their resume, statement of teaching interest and research, and names of three references to: **Faculty Hiring Committee, c/o Ruth Gaus, Qatar Office SMC 1070, 5032 Forbes Avenue, Pittsburgh, PA 15289; Ruth.Gaus@cs.cmu.edu; Fax 412-253-0924.**

- For more information on the Pittsburgh Science of Learning Center, see **http://learnlab.org**.
- For more information on the Human-Computer Interaction Institute, see **http://www.hcii.cs.cmu.edu**.
- For more information on the BS in CS program, see **http://www.csd.cs.cmu.edu/education/bscs/index.html**.
- For more information on the Carnegie Mellon Qatar Campus, see **http://www.qatar.cmu.edu/.**
- Information on Qatar is available at: **http://www.experienceqatar.com/**.

UNIVERSITY OF DELAWARE

One of the oldest institutions of higher education in this country, the University of Delaware today combines tradition and innovation, offering students a rich heritage along with the latest in instructional and research technology. The University of Delaware is a Land-Grant, Sea-Grant, Urban-Grant and Space-Grant institution with its main campus in Newark, DE, located halfway between Washington, DC and New York City. **Please visit our website at www.udel.edu.**

Two Faculty Positions
In Bio–Nanotechnology and Computational & Systems Biology

The Department of Electrical and Computer Engineering (ECE) and the Delaware Biotechnology Institute (DBI), units of the University of Delaware invite nominations and applications for two tenure-track faculty positions in the general areas of: (1) bio-nanotechnology, including active nanostructures, growth and assembly of biomaterials for semiconductors and photovoltaics, and nanodevices with an emphasis on biomedical applications; and (2) computational biology and systems biology. The successful candidates will hold joint appointments in ECE and at DBI and become part of a broader, interdisciplinary collaboration with the College of Engineering. For a full description see our web site **www.ece.udel.edu.**

Applicants should hold a Ph.D. in electrical/computer engineering, biomedical/chemical engineering, or the physical sciences with strong research interests in the life sciences. Successful candidates are expected to have demonstrated excellence in innovative research and show the potential for high quality teaching and mentoring.

Please submit a resume, a statement of research, teaching interests, achievements, and a list of at least four references to either fsearch@ece.udel.edu or Faculty Search Committee, Department of Electrical and Computer Engineering, University of Delaware, Newark, DE 19716. The curriculum vitae and letters of reference shall be shared with departmental faculty. Review of applications will begin immediately and will continue until the position is filled.

The UNIVERSITY OF DELAWARE is an Equal Opportunity Employer which encourages applications from Minority Group Members and Women.

MRC Laboratory of Molecular Biology

MRC Laboratory of Molecular Biology, Cambridge
Postdoctoral Career Development Fellow

A Postdoctoral Career Development Fellow is required for a three year research project, working with Dr Jason W Chin in the Division of Protein and Nucleic Acid Chemistry at the Laboratory of Molecular Biology. The areas of research are Synthetic Biology, Chemical Biology and Engineering New Cellular Function (Rackham and Chin, 2005, Nature Chem. Biol. 1: 159-166).

Applicants should have a PhD degree and experience with molecular biology techniques, preferably the construction of gene libraries, and protein and nucleic acid biochemistry. For further information, please contact: Dr Jason Chin, email: chin@mrc-lmb.cam.ac.uk Applicants for the position are requested to apply at the earliest opportunity, although all applications will be considered up until 17th January 2006.

The starting salary will be in the range of £24,746 - £25,766 per annum (depending on qualifications and experience) supported by a flexible pay and reward scheme and optional final salary pension scheme. We can offer 30 days annual holiday entitlement plus public holidays and excellent sports and social facilities are available on site.

Applications should include a full CV and the contact details of two professional referees who can be approached prior to interview. Please quote job reference PNAC/1105/3 and email to recruit@mrc-centre.cam.ac.uk and copy to Jason Chin, email: chin@mrc-lmb.cam.ac.uk or post to Recruitment Office, Personnel Department, MRC Centre, Hills Road, Cambridge CB2 2QH.

Closing date: 17th January 2006.

For further information about MRC visit www.mrc.ac.uk
The Medical Research Council is an Equal Opportunities Employer.
'Leading Science for Better Health'

WESTFÄLISCHE
WILHELMS-UNIVERSITÄT
MÜNSTER

Interdisciplinary Research Center for Cooperative and Functional Nanoscale Systems

W2-Professor Position

Synthesis and Generation of Functional Nanoscale Systems

to be filled April 1st, 2006

The University of Münster, Faculty of Chemistry and Pharmacy invites highly qualified scientists to apply for a new faculty position at the Institute of Organic Chemistry. The position is part of the new Interdisciplinary Research Center for Cooperative and Functional Systems (FOKUS) which is dedicated to fundamental research in cooperative and functional nanoscale systems.

The aim is the synthesis of novel organic materials. Possible research areas could be: chemistry at interfaces, functional polymers, bio-inspired materials, complex molecular systems, and chemistry in confined systems.

The Collaborations with the existing research groups in Chemistry, Physics, Biology, Pharmacy, Medicine and the Center for Nanotechnology (CeNTech), and interdisciplinary cooperations with existing Collaborative Research Centers (SFB's) and Graduate Colleges are welcome.

Applicants should take an active interest in fostering the development of new, interdisciplinary structures and initiatives. The appointee is expected to teach Organic Chemistry. A participation in academic self-governing of the university is expected as well.

Required qualifications include a strong research record. While these qualifications are generally acquired during a junior professorship appointment or by habilitation, they can be substituted by equivalent expertise earned during postdoctoral research at academic or non-academic institutions inside or outside Germany. Applications from female candidates are particularly encouraged. In the case of equal qualifications of short-listed applicants, preference will be given to physically challenged individuals.

Applications should include a full curriculum vitae, a full list of publications including the reprints of five major scientific contributions, summary of research interests and a future research plan. Applications should be directed to: **Dekan des Fachbereichs Chemie und Pharmazie, Hittorfstraße 58-62, 48149 Münster.**

The closing date for the application is **January 4th, 2006.**

The **Department of Psychological and Brain Sciences** at **Dartmouth College** is seeking applicants for a senior faculty appointment in cognitive neuroscience and to be Director of the Center for Cognitive Neuroscience. The successful candidate is expected to have a distinguished record of accomplishments, including a demonstrated commitment to training students and postdoctoral fellows, as well as a strong potential for sustained external funding. Applications representing any sub-specialization in cognitive neuroscience, broadly defined, are welcome. We are particularly interested in applicants who not only complement our current strengths in memory, cognition, perception and human functional brain imaging, but also make connections to other departmental faculty. The department is housed in a state-of-the-art research and teaching facility that includes a dedicated research MRI scanner for brain mapping research.

Please send a letter of application and a curriculum vitae to: **Dr. Scott Grafton, Chair, Cognitive Neuroscience Search Committee, Department of Psychological and Brain Science, 6207 Moore Hall, Dartmouth College, Hanover, NH 03755**. Informal inquiries regarding the position are also welcome (**scott.grafton@dartmouth.edu**). Review of applications will begin **December 1, 2005** and continue until the position is filled.

With an even distribution of male and female students and over a quarter of the undergraduate student population members of minority groups, Dartmouth is committed to diversity and encourages applications from women and minorities. Dartmouth College is an Equal Opportunity, Affirmative Action Employer.

Faculty Positions

UNMC Eppley Institute for Research in Cancer and Allied Diseases

The **Eppley Institute for Research in Cancer and Allied Diseases**, a multi-disciplinary cancer research institute at the **University of Nebraska Medical Center (UNMC)**, invites applications for tenure-leading positions at all levels. We seek candidates with outstanding record of research achievement with interests relevant to cancer research including, but not limited to: control of cell growth and death, regulation of gene expression, oncogene and tumor suppressor function, tumor immunology, animal models, metastasis and angiogenesis, chemical biology, cancer etiology and chemoprevention. We encourage applications from researchers focusing on basic molecular and cellular mechanisms, as well as those focusing on molecular therapeutics and specific disease models.

The Eppley Institute for Research in Cancer and Allied Diseases, an integral part of both the University of Nebraska Medical Center and the UNMC Cancer Center (NCI-designated Cancer Center), continues aggressive recruitment of outstanding scientists in several areas of scientific priority. The Institute provides a supportive environment that fosters creative, multidisciplinary research with world-class laboratory facilities, state of the art core facilities, and outstanding institutional and state support. New faculty will find a collaborative scientific environment coupled with very competitive start-up packages. Both pre- and post-doctoral fellowships are available for support of trainees. Omaha, the nation's 42nd largest city, offers an outstanding school system, low cost of living, and numerous recreational activities.

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. Applicants can apply online to position # 0831 at **https://jobs.unmc.edu**. Additional information can be found at **http://www.unmc.edu/cancercenter/**. Candidates should also forward a minimum of 3 letters of reference to: **Search Committee, Eppley Institute for Research in Cancer and Allied Diseases, Attn: Matt Winfrey, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, Nebraska, 68198-6805.**

The University of Nebraska Medical Center is an Equal Opportunity Employer.

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ccri

CANCER SCIENTISTS

The **Children's Cancer Research Institute** (**CCRI**) of *The University of Texas Health Science Center at San Antonio* (UTHSCSA) is seeking outstanding candidates (Ph.D., M.D./Ph.D. or M.D.) for research or tenure-track positions at Assistant/Associate/Full Professor levels for its programs in molecular oncogenesis, hematologic malignancies, cancer genetics, and experimental cancer therapeutics. Applicants must have high quality peer-reviewed publications, evidence of independent research and competitive funding potential. The positions offer significant scientific resources and an attractive start-up support package.

The **CCRI** is a unique specialized cancer center, is housed in a new 100,000 sq. foot research facility on the North Campus of UTHSCSA and supported by a $200 million endowment from the tobacco settlement from the State of Texas [refer to http://ccri.uthscsa.edu]. Successful applicants will join a multidisciplinary team of researchers at the **CCRI**. The **CCRI** is a component of the UTHSCSA [refer to www.uthscsa.edu] which is located at the edge of the beautiful Texas Hill Country. San Antonio is the nation's eighth largest city and offers a rich, multi-cultural community with a thriving bioscience industry.

Review is ongoing and continues until positions are filled. Applicants should send current curriculum vitae, a description of research plans, and three letters of reference to:

Sharon B. Murphy, M.D.
Professor & Director

Children's Cancer Research Institute
The University of Texas Health Science Center at San Antonio

MC 7784
7703 Floyd Curl Drive
San Antonio, TX 78229-3900
[210] 562-9003 or murphysb@uthscsa.edu

All faculty appointments are designated as security sensitive positions.
UTHSCSA is an Equal Employment Opportunity/Affirmative Action Employer.

UCL

The Advanced Centre For Biochemical Engineering

Two Staff/Faculty Positions

Regenerative Medicine Bioprocessing

The Centre wishes to make a new academic staff appointment to strengthen further its position in the field of regenerative medicine. Depending on background the new staff member would contribute to building the biology-bioprocess interface by exploring the stem cells used or by definition of the bioprocesses needed. The Centre has superb new facilities for regenerative medicine bioprocessing research and the dozen researchers involved embrace the disciplines of biology, engineering, chemistry and surgery. The achievement of consistent preparation of human cell-based medicines is one of the great challenges for future healthcare.

Bioprocess Microfluidics

The Centre wishes to make a new academic staff appointment to strengthen further its bioprocess research position at the micro-scale. We achieve accurate prediction of large-scale bioprocessing using automated microwell-based methods. Some of the future needs would be particularly well served by also using microfluidic techniques. Though widely applied to chemistry and analytical studies these methods have yet to be explored in relation to the examination of complete bioprocesses. Bioprocess microfluidics poses fundamental challenges and has exciting potential.

The Centre is the principal one in the UK, and probably globally, linking advanced bioscience to an understanding of complete bioprocesses by which discoveries are translated to healthcare outcomes. It has invested over £30 million in new facilities in recent years. From the 1960s it has pioneered studies underpinning the availability of new generations of more complex small molecule drugs made using biocatalysis combined with chemistry. The Centre has also established much to the bioprocess foundation for engineered protein therapy. We have recognised commonalities among our small and macromolecular research activities and begun also to apply these approaches to human cells and engineered tissue. To achieve the desired outcome we collaborate with the best scientists in the field. The dozen staff members co-publish with one another and with colleagues from the life science, physical science and clinical faculties, and a potential staff member must wish to have such collaborations. The Department has 80 doctoral students, a dozen post docs, 30 masters and 120 undergraduate students and teaching embraces the new research territories (www.ucl.ac.uk/biochemeng). The level of appointment will depend on the qualifications and experience of the successful applicant. Current salary ranges (including London Allowance) are; for Lecturer (Assistant Professor) £26,952 to £43,694; Senior Lecturer/Reader (Associate Professor) £41,085 to £49,662. Applications with CV to Miss Tracy Woods via email (t.woods@ucl.ac.uk) or to Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE.

Closing date: 16th January 2006.

RIKEN

RIKEN Research Center for Allergy and Immunology
Position is available for an enthusiastic
RESEARCH COORDINATOR

RCAI

We welcome an energetic and promising coordinator with an international background. For more information on our center, please visit: **http://www.rcai.riken.go.jp/indexE.html**

Functions and Responsibilities

- Annual Report Production
- Annual Advisory Committee Coordination
- International Research Coordination
- Archives, Statistics, & Surveys

Qualification Requirements

- Life sciences background esp. in immunology, ideally PhD
- Editing and writing in English

Working Conditions

Full time, employment contract shall be for one year, renewable annually.

Start of Employment

January 1st, 2006 (negotiable)

Deadline

Application will be closed after a suitable candidate is found.

Selection Process

After selection on the basis of your application, an individual interview will be arranged.

For Application

- CV with a photo
- Publication list with a few reprints of your major publications if available
- Two letters of recommendation
- A letter to state your interest in this position

Send your application to:

Mr. Osamu Ishizuka, Personnel and Welfare Group,
Yokohama Research Promotion Division, RIKEN, 1-7-22
Suehiro, Tsurumi, Yokohama, Kanagawa, Japan 230-0045.
Phone: +81 45 503-9119 Fax: +81 45 503-9112
e-mail: oishizuk@riken.jp

PHILIPPS-UNIVERSITÄT
MARBURG

Professor for Molecular Oncology
(W2 BBesG)

This is a new and tenure track position at the Department of Hematology, Oncology and Immunology (Chair: Prof. Dr. Andreas Neubauer), Philipps University, Marburg, Germany. The successful candidate is expected to head a research group for Molecular Oncology at the department of Hematology, Oncology and Immunology.

The candidate should have an outstanding scientific record and convincing experience as research scientist. In addition, we expect successful grant support from major funding agencies. The candidate should have his/her focus in the field of tumor biology.

This professorship will strengthen the research in molecular oncology at the Philipps University Marburg. The position is equipped with competitive laboratory space at the Institute of Molecular Tumor Biology **(www.imt-uni-marburg.de).** Participation in the Collaborative Research Center "Transregio 17: *Ras signaling in human cancer*", headed by Prof. Dr. Martin Eilers, and other major research activities at the university is expected. In addition, we expect contribution to teaching efforts.

The Philipps University is an equal opportunities employer and applications from minorities and women are encouraged. Qualified handicapped people are also encouraged to apply. Applications with supporting documents (CV, including professional and scientific career; list of scientific publications; copies of the three most important publications; list of successful external grant support; teaching experience; copies of certificates) should be submitted to the **dean of the Medical Faculty, Prof. Dr. Maisch, Baldingerstraße, 35033 Marburg, Germany.**

(deadline: **four weeks** after publication of this advertisement)

POSITIONS OPEN

ASSISTANT PROFESSOR
University of Alberta
Department of Medical Microbiology and Immunology

The Department of Medical Microbiology and Immunology (MMI), University of Alberta seeks applicants for two tenure stream Faculty positions at the Assistant Professor level. Areas of interest include (but are not limited to) research concerning: host-pathogen interactions, molecular pathogenesis, immune responses in model systems, transplant and cancer immunology, and immunogenomics. Applicants must have a proven record of research achievement and be competitive for external salary awards and research grants. Successful candidates will have 75 percent of their time protected for research.

The University is located in the capital of the flourishing province of Alberta and ranks in the top tier of large Canadian universities. MMI offers an environment where researchers can access state-of-the-art infrastructure and find opportunities for collaboration with internationally recognized departmental colleagues (see **website: http://www.ualberta.ca/~mmi).** Remuneration will be commensurate with qualifications and experience. An attractive startup package, including three years of initial salary support, will be provided.

Individuals with a Ph.D. and appropriate postdoctoral training should submit curriculum vitae, a brief description of research plans, and the names of three individuals who may be contacted for letters of reference to:

Medical Microbiology and Immunology Search Committees
c/o Dr. D. Evans, Chair
Department of Medical Microbiology and Immunology
University of Alberta
Edmonton, Alberta, Canada T6G 2H7

The closing date for this competition is February 15, 2006, although these positions will remain open until filled.

All qualified candidates are encouraged to apply; however, Canadian citizens and permanent residents will be given priority. If suitable Canadian citizens and permanent residents cannot be found, other individuals will be considered.

The University of Alberta hires on the basis of merit. We are committed to the principle of equity in employment. We welcome diversity in the workplace and encourage applications from all qualified women and men, including persons with disabilities, members of visible minorities, and Aboriginal persons.

Organismal Biology, ASSISTANT PROFESSOR: The Biology Program of Lynchburg College invites applications for a tenure-track position beginning in the 2006-07 academic year. Candidates should have a Ph.D. degree by the starting date; all but dissertation may be considered. Individuals with broad training in animal biology are encouraged to apply. Responsibilities include teaching introductory biology, animal biology, advanced courses in candidate's specialty, supervising student research projects, continuing scholarly development, student advising, and participating in college service. Teaching experience is desired, and a primary interest in teaching undergraduates at a liberal arts institution is a must. Review of applications will begin December 1, 2005, and continue until the position is filled. Members of Phi Beta Kappa are encouraged to apply.

Send letter of application, curriculum vitae, teaching philosophy as it pertains to a liberal arts science curriculum, statement of research interests, copies of graduate transcripts, and list of three references, along with their e-mail addresses, mailing addresses, and telephone numbers to: **Sally Hargis, School of Sciences, Lynchburg College, 1501 Lakeside Drive, Lynchburg, VA 24501 USA.** We will also accept e-mail applications sent to **e-mail: hargis@lynchburg.edu.** For more information about Lynchburg College, please visit our **website: http://www.lynchburg.edu.**

Lynchburg College is an Equal Opportunity Employer.

POSITIONS OPEN

City of Hope
Where the Power of Knowledge Saves Lives!

ASSISTANT PROFESSOR (TENURE-TRACK)
Radiation Resistance Mechanisms in Cancers
The Department of Radiation Biology
City of Hope/Beckman Research Institute

The Department of Radiation Biology in the Division of Radiation Oncology, together with City of Hope Comprehensive Cancer Center, is seeking outstanding applicants for a tenure-track Assistant Professor position focused in the basic science investigation of fundamental radiation resistance mechanisms in cancer cells or in the relevant areas.

The successful candidate will be expected to develop an independent research program with preference given to candidates using approaches based on biochemistry and molecular genetics (mouse model) to explore fundamental radiation resistance mechanisms in cancer cells, including radiation damage responses, signaling, check points, DNA double strand break repairs and radiation-induced apoptosis. The candidate will also be expected to actively participate in the Cancer Center program(s). As an National Cancer Institute-designated Comprehensive Cancer Center, the City of Hope has excellent shared core-facilities, such as DNA, peptide and RNA synthesis, mass spectrometry, mouse transgenesis, animal care and pathology. The institute also offers the opportunity for interaction with radiation oncologists, medical oncologists, and basic biologists in various areas, including the opportunity to participate in a Ph.D. program in Biological Sciences. A competitive startup package, 12-month institutionally funded salary, and adequate laboratory and office space will be offered to an identified candidate.

The City of Hope is located in one of the most desirable areas of Southern California with easy access to all regional attractions. To learn more about City of Hope and Los Angeles, please visit **website: http://www.cityofhope.org.**

Applicants should send their curriculum vitae, description of research focus, and the names of three references to:

Binghui Shen, Ph.D.
Professor and Director
Department of Radiation Biology
City of Hope National Medical Center and Beckman Research Institute
1500 East Duarte Road
Duarte, CA 91010
E-mail: bshen@coh.org
Website: http://www.cityofhope.org/radbio/

BEHAVIORAL NEUROSCIENCE

The Department of Biology at the University of Kentucky invites applications for a tenure-track **ASSISTANT PROFESSOR** position to investigate the basis of complex processes in neurobiology including, but not limited to, development, plasticity, or behavior. Preference will be given to candidates working in genetically tractable systems using genomic, proteomic, bioinformatics, or quantitative approaches. The successful candidate is expected to develop a vigorous, extramurally-funded research program and participate in undergraduate and graduate instruction. The startup package includes a competitive salary, a generous budget, and an outstanding collegial environment. Send curriculum vitae, research statement, and three reference letters to:

Doug Harrison
Department of Biology
University of Kentucky
101 Morgan Building
Lexington, KY 40506
E-mail: dough@uky.edu

Consideration of applications will begin January 2, 2006. *Equal Opportunity/Affirmative Action Employer. Women and Minorities encouraged to apply.*

POSITIONS OPEN

Biology: Tenure-track position anticipated in Biology at the rank of **ASSISTANT PROFESSOR** starting August 2006. Ph.D. required. Duties will include one section of the Department's required introductory biological principles course (with lab), and elective offerings suitable for students in both of the college's two life-science programs: biology and BBMB (biochemistry, biophysics & molecular biology). An active research program and supervision of undergraduate student research are also expected. We seek applicants with an area of expertise complementary to others in the Department. (Departmental information is available at **website: http://www.whitman.edu/biology**). Examples include, but are not restricted to, evolutionary-developmental biology; cytogenetics; plant pathology or physiology; immunology; or the application of genetics to questions in eukaryotic cell biology. In this search, the college wishes to reinforce its commitment to increase faculty diversity, recognizing that to provide a diverse learning environment is to prepare students for personal and professional success in an increasingly multicultural and global society. In their application, candidates should address how they can contribute to diversity, a core value of the Whitman College community. They should also discuss their interest in working with undergraduates as a teacher and scholar in a liberal arts environment that emphasizes close student-faculty interaction. The successful candidate should demonstrate an interest in participation in the College's general education offerings. Applications will be reviewed in an ongoing basis and should be received by January 16, 2006, to be fully considered by the Search Committee. Applicants should send, as hard-copy: application letter, curriculum vitae, statements of research and teaching interests, college and graduate transcripts (unofficial are acceptable), and three letters of recommendation to: **Tenure-Track Search, Dr. Daniel M. Vernon, Biology Department, Whitman College, 345 Boyer Avenue, Walla Walla, WA 99362.** Whitman College is a top-tier undergraduate institution located in historic Walla Walla, near the Blue Mountains in eastern Washington. For further information about Whitman College, see our **website: http://www.whitman.edu.** *Whitman College is committed to attracting and retaining highly qualified individuals who collectively reflect the diversity of the nation. No applicant shall be discriminated against on the basis of race, national or ethnic origin, age, gender, sexual orientation, marital status, religion, creed, or disability.*

UNIVERSITY OF IOWA
Department of Internal Medicine

The Department of Internal Medicine is seeking research faculty for a position in the Division of Rheumatology at the rank of **ASSOCIATE.** Qualifications include an M.D. or Ph.D. degree in a scientific discipline related to basic immunologic research. Applicants with a documented interest in signaling events that regulate innate or adaptive immune responses are strongly desired. Applicants should have outstanding skills in basic immunology research and signal transduction with clear potential for establishing a funded research program. Salary will be based upon the applicant's qualifications and responsibilities.

Candidates should submit curriculum vitae to:

Paul B. Rothman, M.D.
Chair, Department of Internal Medicine
200 Hawkins Drive, SE308 GH
Iowa City, IA 52242

ASSISTANT PROFESSOR, CELL/DEVELOPMENT BIOLOGY: Applications are invited for a tenure-track position in the Biological Sciences Department at North Dakota State University (NDSU) starting fall 2006. Candidates will be expected to develop an extramurally-funded research program and to teach courses in their area at the undergraduate and graduate levels. For complete description and requirements, see **website: http://biology.ndsu.nodak.edu/** or contact **e-mail: mark.sheridan@ndsu.edu.** Review of applications will begin January 10, 2006. *NDSU is an Equal Opportunity Institution.*

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

FELLOWSHIPS

AAAS 2006 Mass Media Science & Engineering Fellows Program

Are you interested in science writing?

Do you want to help people understand the impact and importance of discovery?

Work to increase public understanding of science and technology.

Fellows in the 10-week 2006 summer program will work as reporters, researchers, and production assistants in mass media organizations nationwide.

2005 host sites included:

Chicago Tribune, The Los Angeles Times, National Public Radio, Scientific American

For eligibility requirements, please visit ***http://ehrweb.aaas.org/massmedia.htm*** for more details and an application brochure, or call 202-326-6441 for more information.

DEADLINE JANUARY 15, 2006

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

UBC

THE UNIVERSITY OF BRITISH COLUMBIA

www.ubc.ca

Centre for Molecular Medicine and Therapeutics

CMMT

Junior, Established and Senior Investigators

The Centre for Molecular Medicine and Therapeutics at the University of British Columbia in Vancouver – "the world's most livable city" - seeks applications for the following three tenure track positions:

British Columbia Leadership Chair in Genetic Medicine – this position will provide a unique opportunity for an internationally recognized individual to perform research in an outstanding environment, positioning the province as a leader in medical genetics. The successful applicant will conduct innovative research that will generate new knowledge relevant to genetic contributions to illness, molecular determinants and mechanisms of disease and development of novel therapeutic approaches and technologies to improve treatment for disease.

Canada Research Chairs – Tier 1 & 2 – these prestigious appointments will provide significant resources for start-up and are aimed at outstanding researchers who are world leaders or have the potential to be world leaders in their fields. The successful candidates' research will interface with scientific programs within the Centre, and will directly address issues of medical relevance. Positions are subject to review and final approval by the CRC Secretariat.

We are especially interested in applicants working in the following fields:

- Genetic variation and susceptibility to disease
- Human genetics
- Statistical genetics
- Bioinformatics
- Pharmacogenetics
- Experimental therapeutics
- Mechanisms of genetic disease

The CMMT has a scientific mandate to ascertain cellular and protein function relevant to human disease as the key to improved diagnosis, treatment and prevention of health problems in children and adults. The CMMT includes outstanding infrastructure support such as a bioinformatics, expression profiling, DNA sequencing, antibody production and world-class facilities for mouse genetics (transgenics, breeding and behavior testing) and the testing of experimental therapeutics in animal models of human disease.

The successful candidates will be appointed as members of the full-time faculty. Applicants should ideally hold an M.D. and/or Ph.D. degree or equivalent and a record of recognized accomplishment in areas relevant to Human Biology and Disease. Salary and rank will be commensurate with qualifications and experiences. These are full-time tenure track appointments and the CRCs are subject to final budgetary approval.

UBC Faculty of Medicine

Anticipated start date for these positions is July 1, 2006. Closing date for all applications is January 15, 2006. Please send CV, names of three references and a brief statement of research interests to: **Cindy Jean, Manager, Human Resources, cjean@cmmt.ubc.ca, CMMT, 980 West 28th Avenue, Vancouver, BC V5Z 4H4. (http://www.cmmt.ubc.ca)**

UBC and its affiliates hire on the basis of merit and are committed to employment equity. We encourage all qualified applicants to apply; however, Canadians and permanent residents of Canada will be given priority. CRCs are open to individuals of any nationality; offers will be made in accordance with the CRC program.

Leading Edge ENDOWMENT FUND

CHILD & FAMILY RESEARCH INSTITUTE

POSITIONS OPEN

FACULTY POSITIONS
Nanoscale Materials
Oklahoma State University

As part of a statewide commitment to excellence in nanoscale science and engineering, Oklahoma State University seeks applicants for two tenure-track assistant professor positions. The successful candidates will be appointed in the Departments of Chemistry or Physics in the College of Arts and Sciences, and/or in the Schools of Chemical, Electrical and Computer, or Mechanical and Aerospace Engineering in the College of Engineering, Architecture and Technology. Oklahoma has existing strengths in polymers at interfaces, carbon nanotubes, fuel cell materials, and sensors. We seek candidates who will expand our strengths in these or other related and complementary areas. Applicants should have an earned Ph.D. in an appropriate discipline. Research experience beyond the Ph.D. is desirable. Successful candidates will be expected to develop an externally funded, internationally recognized research program in nanoscale science and/or engineering; to excel in teaching at both the undergraduate and graduate levels; and to work collaboratively across the university and state. Submit a letter of application, curriculum vitae, descriptions of two research projects with plans to secure external funding, a statement of teaching interests and philosophy, and the names and contact information of five references to: **Nanoscale Materials Search Committee, 201 ATRC, Oklahoma State University, Stillwater OK, 74078.** Enquiries or PDF-formatted applications may be sent to **e-mail: nanosearch@ceat.okstate.edu.** Review of applications will start February 1, 2006, and continue until the positions are filled. The target starting date is August 2006. *Women and minority applicants are encouraged. Oklahoma State University is an Equal Opportunity Affirmative Action Employer.*

Assistant Professor, PLANT VIROLOGIST: The Department of Biological Science at the University of Tulsa seeks to appoint a tenure-track Assistant Professor with expertise in the ecology and evolution of plant viruses, a position funded by a state National Science Foundation Experimental Program to Stimulate Competitive Research initiative. The successful candidate will develop an independent, extramurally-funded research program in plant ecology and evolution and train undergraduate and graduate students. A Ph.D. degree and postdoctoral experience are required. The position begins 15 August 2006. The University of Tulsa is a private, comprehensive university with a strong commitment to research and teaching. The Department of Biological Science has a faculty of fourteen and offers B.S., M.S., and Ph.D. degrees. The Department has excellent research facilities and supporting core labs for DNA sequencing, confocol microscopy and microarray analysis. To apply, send curriculum vitae, statement of research and teaching interests, reprints, and three letters of reference to: **Search Committee Chair, Department of Biological Science, The University of Tulsa, 600 South College Avenue, Tulsa, Oklahoma, 74104.** Review of applications will begin 1 December 2005. *The University of Tulsa is an Equal Opportunity/Affirmative Action Employer.*

CHAIR
Department of Pharmaceutical Sciences

The School of Pharmacy at the Massachusetts College of Pharmacy and Health Sciences, Boston campus, invites applications for the position of Chair, Department of Pharmaceutical Sciences. The successful candidate must have a Ph.D. in pharmaceutical or biomedical sciences and meet the criteria for appointment to the rank of full professor. The Chair will have vision and dedication to develop the department into national prominence.

Review of applications will begin immediately and continue until the position is filled. For details about this position, please visit our **website: http://www.mcphs.edu.** *Equal Employment Opportunity/Affirmative Action Employer.*

TENURE-TRACK ASSISTANT PROFESSOR
in Cell/Molecular Biology
Oakland University
Department of Biological Sciences

The Department of Biological Sciences at Oakland University invites applications for a tenure-track Assistant Professor position to be filled by August 2006. We seek a candidate who will investigate the molecular mechanisms of eukaryotic inter- and intracellular signaling. A Ph.D. and postdoctoral experience are required as well as a strong research track record evidenced by publications. Laboratory space and competitive startup package will be provided. The successful candidate is expected to develop a vigorous, extramurally funded research program, to teach effectively at the undergraduate and graduate levels, and to mentor graduate students in doctoral programs.

The Department of Biological Sciences (**website: http://www2.oakland.edu/biology/**) is a modern, well-equipped, and research-oriented Department of 19 faculty members. Oakland University is a state-supported institution of 17,000 students situated on a beautiful 1,600-acre campus 25 miles north of Detroit.

Review of applications will begin on February 1, 2006, and continue until the position is filled. Applicants should submit curriculum vitae, statement of research plans and teaching philosophy, and key reprints and should have three letters of reference sent to:

Anne L. Hitt, Ph.D.
Search Committee Chair
Department of Biological Sciences
Oakland University
Rochester, MI 48309-4401
E-mail: biology1@oakland.edu

Oakland University is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.

FACULTY POSITION
Synthetic Organic Chemistry

The Department of Chemistry at Portland State University (PSU) invites applications for an open-rank Faculty Position to begin September 2006. The successful applicant will have an established, funded, and independent research program in the application of synthetic organic chemistry to biological problems. Applicants should go to **website: http://www.chem.pdx.edu** for application procedures and information about the University, the Department, and its graduate (Ph.D. and M.S.) programs. Minimum requirements for hire include a Ph.D. and postdoctoral experience, and submission of current curriculum vitae, description of research plans, and funding history. Questions regarding this position can be addressed to: **Dr. S. Reed, Chair of Search Committee, e-mail: sreed@pdx.edu.** Review of applications will begin on January 15, 2006. *PSU is an Affirmative Action/Equal Opportunity institution and, in keeping with the President's diversity initiative, welcomes applications from diverse candidates and candidates who support diversity.*

PLANT BIOLOGIST: Bethel University invites applications for a full-time, tenure-track appointment in the Department of Biological Sciences, beginning fall 2006. Requires Ph.D. in a botanical discipline. Record of successful college teaching preferred. Participation in teaching introductory biology for majors and nonmajors and teaching upper-level courses. Must be strongly committed to the liberal arts educational mission as described at **website: http://www.bethel.edu.** Position is pending final budgetary approval. Letter of application and curriculum vitae addressed to: **Dr. Debra Harless, Dean, 3900 Bethel Drive, St. Paul, MN 55112.**

The Department of Microbiology and Molecular Genetics at Oklahoma State University invites applications for two **ASSISTANT PROFESSOR** positions beginning August 2006. We are seeking individuals addressing fundamental research problems in the areas of molecular microbial pathogenesis and molecular microbial ecology. Competitive startup funds and laboratory space are available. These are nine-month, tenure-track appointments with research, teaching, and service responsibilities. The candidates must have a Ph.D. degree or equivalent and productive postdoctoral experience showing potential for developing and sustaining an independent and extramurally funded research program. The successful candidates are expected to participate actively in graduate and undergraduate teaching and training. Applications should include a letter of interest, a current resume, statements of research goals and teaching philosophy, and three letters of reference. Inquiries and applications should be directed to: **Robert Burnap, Ph.D., Faculty Search Committee Chair, Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078-3020. Telephone: 405-744-7445** or **e-mail: burnap@okstate.edu.** Electronic submissions, preferably in PDF format, may be submitted to **e-mail: rsallie@okstate.edu** with subject line stating **"MMG Faculty Position."** For full consideration, applications should be received by January 30, 2006, but applications will be accepted until the position is filled. *Oklahoma State University is an Equal Opportunity Affirmative Action Employer. Women and Minorities are strongly encouraged to apply.*

RESEARCH ASSISTANT PROFESSOR
University of North Texas Health Science Center
Cancer Biology

The Department of Molecular Biology and Immunology invites applications for a full-time appointment at the rank of Research Assistant Professor in the area of cancer biology. Candidates must hold a Ph.D. and relevant postdoctoral experience with a strong record of excellence in research. The start date is negotiable. Although the research area in cancer biology is open, preference will be given to applicants with significant experience in animal models. The successful candidate will be expected to develop and expand an independent, externally funded research program. The individual is also expected to participate in teaching both graduate and medical students.

To insure full consideration, please apply online at **website: https://www.unthscjobs.com/applicants/jsp/shared/frameset/Frameset.jsp** and submit the following material: (1) current curriculum vitae and list of publications and grant support; (2) brief statement of research interests; and (3) contact information for three references no later then November 30, 2005.

The University of North Texas Health Science Center is an Equal Opportunity/Affirmative Action Employer. Hiring is contingent upon eligibility to work in the United States. Women and Minorities are especially encouraged to apply.

RESEARCH FACULTY
Behavioral Neuroscience

Stony Brook University's Department of Psychiatry invites applicants with a Ph.D. or equivalent for a position at the level of Research Assistant or Associate Professor. Applicants should have demonstrated expertise in visuomotor, circadian, or sleep regulatory systems with use of electrophysiological recording methods. The successful candidate should be willing to collaborate with an existing laboratory studying biological rhythm regulation, the vestibular system, and non-image forming visual system, while establishing a fully independent laboratory with his/her own research grant support. Send curriculum vitae, statement of specific research interests, reprints or preprints of completed research, and three recommendation letters to: **Mark J. Sedler, M.D., MPH, Chairman, Department of Psychiatry, HSC T10-020, Stony Brook University, Stony Brook, NY 11794-8101.** Visit **website: http://www.stonybrook.edu/cjo** for employment information. *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

FACULTY POSITION
Department of Applied Science
College of William & Mary

The Department of Applied Science at the College of William & Mary, an interdisciplinary Ph.D.-focused department established in 1995, invites applicants for a tenure-track position at the Assistant Professor level in biophysics, neurophysiology, biomedical engineering, biomaterials, or a related field, emphasizing either computational or experimental approaches. The new faculty member will be expected to establish a vigorous, independent, and well-funded graduate research program at the interface of the physical, mathematical, and biological sciences. Excellence and high commitment to the teaching of graduate and undergraduate students is also expected of all faculty at the College. Located two hours south of Washington, D.C. in Williamsburg, Virginia, the College of William & Mary is the second oldest university in the United States and was recently named by the editors of Newsweek as the "hottest small state school" in the nation. Candidates should submit a complete curriculum vitae, contact information for three letters of reference, and copies of no more than five refereed publications to: **Faculty Search Committee, Department of Applied Science, The College of William & Mary, P.O. Box 8795, Williamsburg, VA 23187-8795.** Review of materials is expected to begin January 1, 2005, and continue until the position is filled. For more information see **website: http://as.wm.edu.** *The College is an Equal Employment Opportunity/Affirmative Action Employer.*

BIOCHEMISTRY: ASSISTANT PROFESSOR, TENURE-TRACK POSITION. The Department of Chemistry and Biochemistry at the University of Tulsa invites applications for a tenure-track position beginning August 2006. A Ph.D. in biochemistry, commitment to quality undergraduate teaching and research, and evidence of teaching and research abilities or potential are required. Postdoctoral experience is preferred. The ten-member department offers American Chemical Society-approved B.S. chemistry and biochemistry degrees. The applicant's research should complement our M.S. programs in chemistry and biochemistry and be able to take advantage of our excellent facilities and instrumentation. See **website: http://www.chemistry.utulsa.edu.** Applicants should submit curriculum vitae, both undergraduate and graduate transcripts, a statement of research plans, teaching philosophy, and three letters of recommendation to: **Dr. Dale Teeters, Search Committee Chair, Department of Chemistry and Biochemistry, The University of Tulsa, 600 S. College Avenue, Tulsa, OK 74104-3189.** Applications will be reviewed beginning January 20, 2006, and continue until the position is filled. *The University of Tulsa is an Equal Opportunity/Affirmative Action employer. Women and minorities are encouraged to apply.*

ASSISTANT/ASSOCIATE PROFESSOR OR PROFESSOR
National Sun Yat-Sen University

The Department of Biological Sciences is seeking candidates for a tenure-track Faculty Position in the area of plant physiology. Candidates for this position must have an interest in teaching and research in the fields of plant physiology, plant tissue culture, plant cell biology and plant molecular biology.

Requirements: Hold a Ph.D. degree in related fields, have postdoctoral research experience, and must be able to communicate and teach in Mandarin.

Applicants should submit statement of career goals, resume, graduate transcript, publication reprints, and three letters of recommendation by January 31, 2006, to:

Dr. Jong-Kang Liu
Chairman
Department of Biological Sciences
National Sun Yat-Sen University
No. 70 Lien Hai Road
Kaohsiung City, TAIWAN 80424

USDA Agricultural Research Service
www.ars.usda.gov

RESEARCH LEADER (Interdisciplinary)
Supervisory Research Plant Physiologist, Supervisory Research Geneticist (Plants) or Supervisory Research Plant Pathologist or Supervisory Research Entomologist

The United States Department of Agriculture, Agriculture Research Service (USDA/ARS), Horticultural Crops Research Unit, Corvallis, Oregon, invites applications for the permanent, full-time position of Research Leader, GS-14/15 (salary range $85,123 to $130,173 per annum, commensurate with experience). The Research Leader will direct a dynamic group of 16 scientists and 50 support staff, and conduct original research evaluating the physiology, genetics, pathology, or arthropod pests of small fruit or nursery crops. United States citizenship is required. For details and application directions, see **website: http://www.afm.ars.usda.gov/divisions/hrd/vacancy/resjobs/x6w-0014.pdf.** To have a printed copy mailed, call **telephone: 541-738-4002.** Applications must be received by December 30, 2005. *USDA/ARS is an Equal Opportunity Employer and Provider.*

FULL-TIME TENURE TRACK ASSISTANT PROFESSOR POSITION
Applied Forensic Sciences Department

Mercyhurst College of Erie, Pennsylvania is seeking highly qualified candidates for a full-time Tenure-Track Position in the Applied Forensic Sciences Department beginning September 2006. Minimum requirements include a Master's degree (Ph.D. strongly preferred) in a forensic science discipline, extensive experience as a forensic scientist, and credentials as a court-qualified expert witness. Field of specialty is open. Duties include teaching undergraduate introductory and advanced courses, conducting professional research and cases, and curriculum development. Applicant must submit a letter of application and current curriculum vitae. Review of applications begins December 1, 2005, and will continue until the position is filled. Contact: **Dr. Dennis C. Dirkmaat, Applied Forensic Sciences Department, Mercyhurst College, 501 East 38th Street, Erie, PA 16546. E-mail: ddirkmaat@mercyhurst.edu.** *Mercyhurst College is an Equal Opportunity/Affirmative Action institution.*

FACULTY POSITIONS

The Department of Cell Biology and Physiology at the University of Pittsburgh School of Medicine invites applications for Tenure-Track Positions at all professorial levels. Departmental research strengths include: epithelial cell biology, regulation of membrane traffic of proteins and lipids and the regulation of gene expression and signal transduction in endocrine systems. We seek individuals whose research will interface with and extend the existing strengths of the Department in these areas. Space and startup funds will be provided by the Department of Cell Biology and Physiology.

Applicants should have a Ph.D. and/or M.D. degree and postdoctoral experience. Send curriculum vitae, summary of research interests and names of three references to: **Raymond A. Frizzell, Ph.D., Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, S368 Biomedical Science Tower, 3500 Terrace Street, Pittsburgh, PA 15261.** *The University of Pittsburgh is an Equal Opportunity/Affirmative Action Employer.*

Biochemistry: Pacific University in Forest Grove, Oregon, invites applications for a full-time, **TENURE-TRACK POSITION** beginning August 2006. Position description and application directions are at **website: http://www.chem.pacificu.edu/biochemsearch/.** Application review begins January 4, 2006. *Women and Minorities are especially encouraged to apply. Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE PROFESSOR
University of Colorado Health Sciences Center at Fitzsimons
Cancer Biology
Department of Craniofacial Biology
University of Colorado Comprehensive Cancer Center

The University of Colorado Comprehensive Cancer Center and the Department of Craniofacial Biology at the University of Colorado School of Dentistry invite applications for a full-time tenure-eligible position in cancer biology at the Assistant or Associate Professor level, commensurate with experience and accomplishments. Applicants should have a Ph.D., M.D. or equivalent degree, postdoctoral research experience in cancer biology or a related field, and an exceptional record of research accomplishments. Individuals with experience in the area of cancer biology including malignant transformation, cell proliferation, signal transduction, cell motility and migration, metastasis, and apoptosis are especially encouraged to apply. The successful applicant will have a joint appointment within a suitable Department within the School of Medicine or the School of Pharmacy. He or she will be expected to develop a vigorous externally funded research program and contribute to the teaching mission at the School of Dentistry and the Graduate School. Applicants should submit a curriculum vitae, brief description of research interests, and arrange to have three letters of reference forwarded by mail or e-mail to the address listed below, **Attn: CBR Project Coordinator**. For full consideration, completed applications should be received by January 15, 2006.

Completed materials for all positions should be sent to: **Dr. Barbara Zimmerman, Department of Craniofacial Biology, University of Colorado Denver Health Sciences Center, Mailstop 8120, P.O. Box 6511, Aurora, CO 80045. (FEDEX address: 12801 E. 17th Ave, Rm. L18-11109, Aurora, CO 80010). E-mail: barbara.zimmerman@uchsc.edu.**

For more information see: **websites: http://www.uchsc.edu/sod/units/research/researchHome.html** and **http://www.uccc.info.**

The University of Colorado is Committed to Diversity and Equality in Education and Employment.

EMPLOYMENT OPPORTUNITY
Des Moines University
Osteopathic Medical Center

FACULTY POSITION, Microbiology Department: Participate in college or university courses for students, conduct research or other scholarly/creative endeavors, and participate in academic governance/service responsibilities.

Minimum Qualifications: (1) An earned doctorate from an accredited university in a relevant field. (2) Must have the desire and potential to become an excellent educator. (3) Must have the desire and potential for successful independent scholarly activity. (4) The employee must occasionally lift and/or move up to 25 pounds.

Des Moines University
3200 Grand Avenue
Des Moines, IA 50312
Website: http://.dmu.edu/employment

Posted November 15, 2005. *An Equal Opportunity Employer.*

Two **POSTDOCTORAL FELLOWS** to study Rickettsial transformation and pathogenesis. Applicants with experience in bacterial pathogenesis, molecular biology and genomics are invited to apply. Submit resume and three references to:

Dr. Abdu F. Azad
Department of Microbiology and Immunology
University of Maryland
School of Medicine
HH Building Room 324
Baltimore, M.D. 21201
E-mail: aazad@umaryland.edu

POSITIONS OPEN

VISITING ASSISTANT PROFESSOR
Ecology

The Department of Biology, Hamilton College, invites applications for a one-year visiting assistant professorship, effective August, 2006. Ph.D. preferred; teaching experience expected. The successful applicant will teach: (1) part of a team-taught course in introductory biology; (2) part of a team-taught course in ecology, (3) an introductory course in environmental science, and (4) a seminar, perhaps in animal behavior or conservation biology, that complements the department's offerings. Support is available for research. Send curriculum vitae, a statement about teaching, and names of three references to: **Ernest H. Williams, Chair, Department of Biology, Hamilton College, 198 College Hill Road, Clinton, NY 13323-1292.** Review of application materials will begin February 1, 2006, and continue until the position is filled. *Women and members of minority groups are encouraged to apply. Hamilton College is an Affirmative Action, Equal Opportunity Employer and is committed to diversity in all areas of the campus community.*

FACULTY POSITIONS
Tenure Track
Department of Psychiatry

The Department of Psychiatry and the Center for the Study of Traumatic Stress at the Uniformed Services University of the Health Sciences (USUHS) seeks to fill tenure-track neuroscience laboratory research and teaching positions (**ASSISTANT/ASSOCIATE PROFESSOR**). The Department, twenty full-time faculty, seeks to expand ongoing neuroscience research, animal and human, in: stress; anxiety (particularly acute stress responses, PTSD and dissociation); depression; behavior and drug use. Individuals who hold Ph D. or M.D. degrees and have active fundable research are invited to apply. Send curriculum vitae, description of current and anticipated research and three references to: **Robert Ursano, M.D., Chairman, Department of Psychiatry, USUHS, 4301 Jones Bridge Road, Bethesda, MD 20814-4799. E-mail: rursano@usuhs.mil.** Review of applications will begin on January 2, 2006. *The University is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION at the University of Chicago is available to study urban particulate matter- mediated airway and cardiac inflammation, toxicology and toxicogenomics using cell culture and animal models. This is a newly funded Environmental Protection Agency project with support for candidates with Ph.D. and/or M.D. degree with experience in lung/airway or cardiac biology, molecular biology, biochemistry and animal models of lung/cardiac injury.

Interested applicants should send or e-mail their research experience, curriculum vitae, and contact information for three references to: **Drs. Joe G.N. Garcia/V. Natarajan, Department of Medicine, University of Chicago, CIS Building, Room # 408B, 929, E. 57th Street, Chicago, IL 60637. E-mail: vnataraj@medicine.bsd.uchicago.edu.**

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.

The Fred Hutchinson Cancer Research Center is seeking a **STAFF SCIENTIST/SCIENTIFIC PROGRAMMER** (Job G-016684). Work with biology laboratory and software development team to evaluate large datasets from mass spectrometry instrumentation. Requirements: Ph.D. in biochemistry or related biological science field; one year graduate level coursework in object-oriented programming using C++, including experience with Java; and two years of data analysis of large-scale quantitative proteomics by liquid chromatography/mass spectrometry (LC-MS) and liquid chromatography/mass spectrometry/mass spectroscopy (LC-MS/MS). Full-time position, Seattle, WA. Salary depending on experience. For more information and/or to apply online, go to **website: http://www.fhcrc.org.**

POSITIONS OPEN

HIGH-THROUGHPUT MOLECULAR BIOLOGY AND PROTEIN PRODUCTION
New York Consortium on Membrane Protein Structure
New York Structural Biology Center

Three positions are available in the New York Consortium on Membrane Protein Structure (NYCOMPS), an National Institutes of Health Protein Structure Initiative (PSI) Center. The NYCOMPS goal is to produce high-resolution 3-D structures of integral membrane proteins on a genomic scale. NYCOMPS is developing a high-throughput automated platform for cloning, expression testing, and protein production for this effort. We are soliciting applications for the following positions:

SENIOR SCIENTIST, Molecular Biology. The candidate, holding a Ph.D. in molecular biology or a similar discipline, will participate in the design and management of a core high-throughput molecular biology facility. Knowledge of protein expression in prokaryotic and eukaryotic systems is desired.

SCIENTIST, Lab Robotics Management. The candidate, with at least a B.A. in a scientific discipline, and a working knowledge of molecular biology, will manage and maintain robotics in the core facility. Excellent organizational skills are required, and basic knowledge of database management is preferred.

SCIENTIST, Protein Expression and Screening. The candidate, with a B.S. in biochemistry at minimum, will perform protein expression, screening, and purification in the high-throughput automated Center labs. Work will be carried out at the NYCOMPS labs at the New York Structural Biology Center (NYSBC), located in New York City (**website: http://www.nysbc.org.**).

Interested individuals should send a cover letter, curriculum vitae, and provide the names of three references. Please contact: **Dr. Jeffrey Bonanno, New York Structural Biology Center (NYSBC), 89 Convent Avenue, New York, NY 10027.** Applications can also be submitted electronically to **e-mail: jbonanno@nysbc.org.** *NYSBC is an Equal Opportunity Employer.*

FACULTY POSITION
Bacterial Pathogenesis

An Assistant/Associate Professor tenure-track position is available for candidates with research interests in the molecular basis of bacterial pathogenesis. Applicants should have a Ph.D. and/or M.D. with relevant postdoctoral experience. Candidates for Assistant Professor will be judged on their potential to develop a vigorous independent research program that can attract extramural support. Applicants to be considered for Associate Professor must have a significant publication record and extramural support. Candidates will also be expected to participate in teaching medical and graduate students as well as supervise dissertation research. An attractive startup package and excellent facilities will be available to the successful candidate. Minority and female candidates are encouraged to apply.

Submit curriculum vitae, statement of research interests, and a list of three references to:

Dr. Paul C. Montgomery, Professor and Chair
Department of Immunology and Microbiology
Wayne State University
School of Medicine
540 E. Canfield Avenue, Detroit, MI 48201

ONE-YEAR ASSISTANT RESEARCH SCIENTIST POSITION

Assemble and assist teams of faculty of the Gulf Coast Cooperative Ecosystem Studies Unit (CESU) to pursue project funding from federal partners. Doctorate required, writing experience preferred. To view the position description or to apply, go online at **website: http://greatjobs.tamu.edu** using Vacancy 01471, by December 15, 2005. Send questions to **e-mail: rdbrown@tamu.edu.**

POSITIONS OPEN

POSTDOCTORAL FELLOW IN POPULATION BIOLOGY: The Center for Population Biology (CPB) at University of California, Davis (UCD) invites applications for a Postdoctoral Fellowship in population biology, broadly defined to include ecology, systematics, population genetics, and evolution. The position is for two years, subject to review after one year, and can begin as early as 1 July 2006. Annual salary is $35,000 plus benefits, and $4,000 per annum in research support. The Fellow will be a fully participating member in the Center for Population Biology, and will be expected to have an independent research program that bridges the interests of two or more CPB laboratory groups. For more information about UCD programs in population biology, see **website: http://www.cpb.ucdavis.edu.** Interested candidates should submit a cover letter, curriculum vitae, a short (one to two page) description of research accomplishments, and a short (one to two page) description of proposed research indicating potential faculty mentors, and copies of two publications at **website: <http://www2.eve.ucdavis.edu/jobs/>http://www2.eve.ucdavis.edu/jobs/,** all as PDFs. You should also have three letters of reference sent to **e-mail: smaceygallow@ucdavis.edu.** Please follow instructions on the website. Application evaluation will begin on January 13, 2006. *The University of California is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to the development of a climate that supports equality of opportunity and respect for differences.*

The University of Kansas Center for Research seeks a Transgenic Gene Knockout **LABORATORY DIRECTOR/SCIENTIST.** Required qualifications: Ph.D. in a relevant biomedical science such as biochemistry, molecular biology, pharmacology or toxicology; substantial expertise in molecular biology, molecular genetics or related field; demonstrated ability for effective communication and interpersonal relations; experience with cloning techniques, genomic library screening, construction of DNA vectors, and ES cell transfection and/or genotyping. Full description at **website: https://jobs.ku.edu.** Send application materials to: **Angie Loving, Kansas University Center for Research, 108 Youngberg Hall, 2385 Irving Hill Road, Lawrence, KS 66045. E-mail: aloving@ku.edu.** Reviews begin February 1, 2006, and will continue until filled. *Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION available to investigate the role of multiple activities of p300/CBP in cell cycle in normal and cancer cells. See **Kolli et al.,** *Proceedings of the National Academy of Sciences (PNAS),* **98:** 4646, 2001; **Baluchamy et al.,** *PNAS,* **100:** 9524, 2003; **Rajabi et al.,** *Journal of Biological Chemistry,* **280:** 361, 2005. Contact: **Bayar Thimmapaya, Professor, Microbiology-Immunology Department, Northwestern University Medical School, Chicago, Illinois. Telephone: 312-503-5224. E-mail: b-thimmapaya@northwestern.edu.**

COURSE

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For more information contact: **Maura Hofstadter, e-mail: mhofstad@uci.edu, telephone: 949-824-3993.**

STANFORD UNIVERSITY
Climate Science Faculty Position

The School of Earth Sciences and the Stanford Institute for the Environment solicit applications for a tenure-track faculty appointment in the area of climate science. We are looking for a person with a demonstrated research record who is also committed to quality undergraduate and graduate teaching. This position will be jointly held between the School of Earth Sciences and the newly formed Institute for the Environment.

We seek a climate scientist who uses models to define and address questions about the structure, function, and expression of the ocean-atmosphere-land-ice system. A commitment to collecting, analyzing, or interpreting climate observations from the past or present will be an asset. The successful applicant will interact effectively with a broad range of Stanford colleagues, including physical, chemical, and biological scientists as well as engineers and social scientists interested in policy implications. The search is open to applicants at all levels.

The position will remain open until filled. Applications, including a curriculum vitae, a statement outlining research and teaching interests, and the names and addresses of three or more referees, should be sent by February 1, 2006, to:

Climate Science Search Committee
School of Earth Sciences
101 Mitchell Building
397 Panama Mall
Stanford University
Stanford, CA 94305

Questions can be directed to **Prof. Robert Dunbar (dunbar@stanford.edu)** or **Prof. Christopher Field (cfield@globalecology.stanford.edu)**.

Stanford University has a strong institutional commitment to the principle of diversity. In that spirit, we particularly encourage applications from women, members of ethnic minorities, and individuals with disabilities.

Genetics/Genomics and Plant Biology Tenure-Track Positions

The Department of Biology at the University of Minnesota Duluth (UMD) invites applications for up to two tenure-track Assistant Professor positions in Genetics/Genomics and Plant Biology starting in August 2006. **Genetics/Genomics:** we seek a person with postdoctoral research experience in genetics or genomics of either prokaryotic or eukaryotic organisms, who will instruct courses in genetics, microbiology or cell biology, and an advanced course in their research specialty. **Plant Biology:** we seek an individual with postdoctoral research experience in plant physiology or developmental biology, who will participate in the core curriculum, instruct a plant physiology or plant developmental biology course, and an advanced course in their specialty area. The successful candidates for both positions will establish an independent, externally funded research program involving graduate and undergraduate student researchers. Opportunities exist for collaboration with researchers at the UMD School of Medicine, the College of Pharmacy, Large Lakes Observatory, EPA Mid-Continent Research Laboratory, and the UMD Natural Resources Research Institute. State-of-the-art research and instructional facilities and competitive startup funding are available. Abundant recreational opportunities and a high quality of life complement the thriving intellectual and artistic atmosphere in the region.

Essential qualifications include a Ph.D. or equivalent degree in the biological sciences; at least one peer-reviewed publication; evidence of potential for achievement in teaching and research; strong oral and written communication skills. Review of complete applications will begin on **January 9, 2006**, and continue until the position is filled. Send letter of application, curriculum vitae, brief statements of teaching philosophy and proposed future research, up to three refereed publications and arrange to have three letters of reference sent to: **Chairperson, Genetics/Plant Biology Search Committee, Department of Biology, University of Minnesota Duluth, 207 SSB, 1035 Kirby Drive, Duluth, Minnesota 55812**. Visit UMD at **www.d.umn.edu/biology** and the Integrated Biosciences graduate program at **www.d.umn.edu/ibs**.

The University of Minnesota is an Equal Opportunity Educator and Employer.

Faculty Position

The Department of Biomedical Sciences at Baylor College of Dentistry, Texas A&M University System Health Science Center, Dallas, is seeking outstanding candidates for full-time faculty positions at the assistant, associate or full professor level for either the tenure or nontenure track. A PhD in physiology or a related science area is preferred, although consideration will be given to any person holding a PhD who has substantial experience teaching physiology. The successful candidate will participate primarily in a team-taught physiology course to first year dental students and graduate students. Applicants should have a broad system-based knowledge of physiology and/or endocrinology. For the tenure track position, applicants must have a demonstrated ability to establish an independent research program and procure extramural funding. Current departmental research strengths include inflammation/pain and craniofacial biology; specifics can be seen at www.bcd.tamhsc.edu. Applications will be reviewed as they are received and the search will continue until the position is filled.

Please submit a curriculum vitae, summary of current research activities, statement of career goals and teaching philosophy, and the names and contact information of at least three individuals for letters of recommendation to: **Dr. Brendan Wong, Search Committee Chair, Department of Biomedical Sciences, Baylor College of Dentistry, TAMUSHSC, 3302 Gaston Avenue, Dallas, TX 75246; Email: bwong@bcd.tamhsc.edu**.

Baylor College of Dentistry is an Affirmative Action/Equal Opportunity Employer committed to excellence through diversity.

USDA ARS Agricultural Research Service
www.ars.usda.gov

Research Geneticist (Plants) GS-12/13

The USDA, Agricultural Research Service, Plant Genetics Research Unit in Columbia, Missouri, is seeking an innovative scientist. Salary is commensurate with experience ($60,576 - $93,643). Ph.D. is desirable. The research is to provide solutions to problems limiting maize productivity and utilization. The applicant is expected to address the genetic bases of specific traits of national concern, conduct experiments that enhance our knowledge of fundamental genetic mechanisms controlling maize productivity such as heterosis, disease and insect resistance, and plant response to abiotic stress, and also to improve the methodology of complex trait analysis in maize. The scientist is expected to provide leadership within teams on aspects of quantitative genetics, agronomic trait evaluation, and utilization of diverse germplasm. United States citizenship is required.

For information on the research program and/or position, contact **M. J. Oliver, Research Leader; tel. 573/882-9645; e-mail olivermj@missouri.edu**. Refer to **http://www.ars.usda.gov/Careers/Careers.htm** for further information and the full text announcement (**ARS-X6W-0032**). Applications must be postmarked by **January 16, 2006**.

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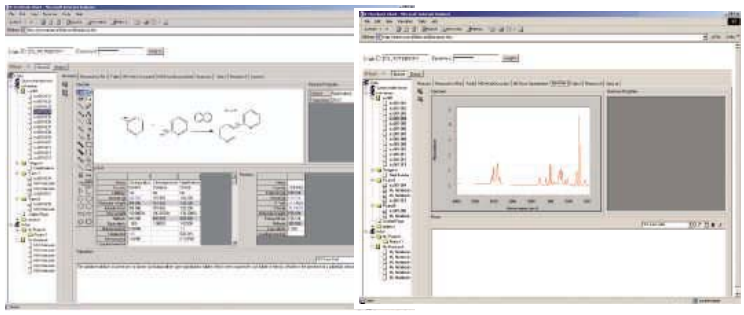

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